# ASSESSMENT AND MANAGEMENT OF THE SOIL-BORNE DISEASE OF SAGE AND LAVENDER IN NORTH-CENTRAL FLORIDA

By

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Dedicated to the memory of my parents,
José Rafael Espaillat G. and Zoila Luz Muñoz
de Espaillat. A special recognition is extended to my wife
Rosemary Vega de Espaillat. Lastly, but very importantly,
I dedicate this research and degree to my children,
José Arturo and Nicole Marie. The love and
marvel of my children made possible my
completion of the Ph.D. program.

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# TABLE OF CONTENTS

page

ACKNOWLEDGMENTS.................................. i

LIST OF TABLES................................... v

LIST OF FIGURES.................................. viii

ABSTRACT......................................... x

CHAPTERS

I INTRODUCTION.................................. 1

II LITERATURE REVIEW............................. 6

Herbs, Spices, Medicinal Plants and Agronomy.. 6
Biosynthesis of Essential Oils and the Environmental Conditions.................... 10
Chemotaxonomy of Sage and Lavender............ 15
  Sage Chemotype.............................. 16
  Lavender Chemotype.......................... 19
Diseases Caused by Soilborne Fungi in Herbs... 22
  Disease Caused by *Fusarium oxysporum* in Sage and Lavender.............................. 26
Disease Caused by *Meloidogyne* spp. on Herbs... 27
  *Meloidogyne* spp. on Sage and Lavender....... 31
Interaction between *Fusarium* spp. and *Meloidogyne* spp............................ 32
Soil Management Practices for Soilborne Disease Control............................ 33
  Cultural Practices.......................... 36
  The Use of Organic Mulches.................. 41

III ASSESSMENT OF PATHOGENS IDENTITY AND PATHOGENICITY................................. 47

Introduction.................................. 47
Materials and Methods......................... 49
  Isolation of *Fusarium oxysporum*........... 49
  Preliminary Pathogenicity Test............. 52
  Differential Host Test..................... 53

Results and Discussion........................ 54
Preliminary Pathogenicity Test with Sage... 54
Preliminary Pathogenicity Test with lavender.................................. 56
Differential Host Test..................... 58

IV COMBINED PATHOGENICITY TESTS WITH *Fusarium oxysporum* AND *Meloidogyne incognita* RACE 1 ON SAGE............................................... 60

Introduction.................................. 60
Materials and Methods......................... 62
Preparation of inoculum.................... 62
Data Collection............................ 65
Statistical Analysis....................... 67
Results and Discussion........................ 68
Measurements over Time..................... 68
First Destructive Sample................... 73
Final Harvest.............................. 77

V COMBINED PATHOGENICITY TESTS WITH *Fusarium oxysporum* AND *Meloidogyne incognita* RACE 1 ON LAVENDER........................................... 83

Introduction.................................. 83
Materials and Methods......................... 85
Data Collection............................ 88
Statistical Analysis....................... 90
Results and Discussion........................ 90
Measurements over Time..................... 90
Non-destructive sample: 123 days........... 91
Harvest Data............................... 98

VI DISEASE CONTROL STUDY WITH SAGE............... 103

Introduction.................................. 103
Materials and Methods......................... 106
Experimental Design........................ 107
Data Collection............................ 111
Statistical Analysis....................... 113
Results and Discussion........................ 113

VII DISEASE CONTROL STUDY WITH LAVENDER........... 129

Introduction.................................. 129
Materials and Methods......................... 132
Experimental Design........................ 133
Data Collection............................ 137
Statistical Analysis....................... 140
Results and Discussion........................ 140

VIII GENERAL DISCUSSION AND SUMMARY................ 152

Pathogenicity Studies......................... 159
Combined Pathogenicity Test with Sage...... 160
Combined Pathogenicity Test with Lavender.. 160
Disease Control Studies....................... 161
Sage........................................ 161
Lavender.................................... 162

APPENDICES

A GERMPLASM USED IN THE RESEARCH ............... 158
B PROCEDURES USED IN THE LABORATORY............. 160
C RATING SCALES USED TO EVALUATE PATHOGEN EFFECTS........................................ 165
D DATA COLLECTED IN THE COMBINED PATHOGENICITY TESTS WITH SAGE............................... 168
E DATA COLLECTED IN THE COMBINED PATHOGENICITY TESTS WITH LAVENDER........................... 172
F DISEASE CONTROL STUDY WITH SAGE............... 175
G DISEASE CONTROL STUDY WITH LAVENDER........... 185

REFERENCES........................................... 201

BIOGRAPHICAL SKETCH.................................. 219

# LIST OF TABLES

TABLE PAGE

3.1 Analysis of variance summary and single degree of freedom contrasts for the preliminary pathogenicity test with sage.................................... 55

3.2 Analysis of variance summary and single degree of freedom contrasts for the preliminary pathogenicity test with lavender................................ 56

3.3 Response of differential host plants to the root-knot nematode isolated from diseased sage plants... 59

4.1 Means and ANOVA summary of the effects of *Fusarium oxysporum* and *Meloidogyne incognita* inoculum levels on the percent dead plants in the first pathogenicity test with sage...................... 69

4.2 Means and ANOVA summary of the effects of *Fusarium oxysporum* and *Meloidogyne incognita* inoculum levels on the cumulative percent dead plants in the second pathogenicity test with sage...................... 70

4.3 Means and ANOVA summary of the effects of *Fusarium oxysporum* and *Meloidogyne incognita* inoculum levels on plant height in the first pathogenicity test with sage......................................... 71

4.4 Means and ANOVA summary of the effects of *Fusarium oxysporum* and *Meloidogyne incognita* inoculum levels on plant height in the second pathogenicity test with sage......................................... 72

4.5 Treatment means and analysis of variance summary for agronomic and *Meloidogyne incognita* data collected in the first destructive sample of the first pathogenicity test with sage................ 74

4.6 Treatment means and analysis of variance summary for *Fusarium oxysporum* data collected in the first destructive sample of the first pathogenicity test with sage......................................... 75

4.7 Treatment means and analysis of variance summary for additional data collected at 123 days after establishment of the second pathogenicity test with sage........................................ 76

4.8 Summary of treatment means and analysis of variance for agronomic, *Fusarium oxysporum* and *Meloidogyne incognita* variables in the first pathogenicity test with sage at harvest.............................. 78

4.9 Summary of treatment means and analysis of variance for agronomic, *Fusarium oxysporum* and *Meloidogyne incognita* variables in the second pathogenicity test with sage at harvest......................... 79

5.1 Means and ANOVA summary of the effects of *Fusarium oxysporum* and *Meloidogyne incognita* inoculum levels on the percent dead plants in the first pathogenicity test with lavender.................. 92

5.2 Means and ANOVA summary of the effects of *Fusarium oxysporum* and *Meloidogyne incognita* inoculum levels on the percent dead plants in the second pathogenicity test with lavender.................. 93

5.3 Means and ANOVA summary of the effects of *Fusarium oxysporum* and *Meloidogyne incognita* inoculum levels on plant height in the first pathogenicity test with lavender.................................... 94

5.4 Means and ANOVA summary of the effects of *Fusarium oxysporum* and *Meloidogyne incognita* inoculum levels on plant height in the second pathogenicity test with lavender.................................... 95

5.5 Treatment means and analysis of variance summary for additional agronomic data collected at different dates after establishment of the first pathogenicity test with lavender.................. 96

5.6 Treatment means and analysis of variance summary for additional agronomic data collected at different dates after establishment of the second pathogenicity test with lavender.................. 97

5.7 Summary of treatment means and analysis of variance for agronomic, *Fusarium oxysporum* and *Meloidogyne incognita* variables in the first pathogenicity test with lavender at harvest..................... 99

5.8 Summary of treatment means and analysis of variance for agronomic, *Fusarium oxysporum* and *Meloidogyne* *incognita* variables in the second pathogenicity test with lavender at harvest...................... 100

6.1 Analysis of variance summary of the plant height data collected over time in the disease control study with sage.................................... 114

6.2 Analysis of variance summary of percent dead plant data collected over time in the disease control study with sage............................ 117

6.3 *Fusarium oxysporum* population density in the soil at harvest............................................ 122

6.4 Analysis of variance summary of the effect of *Fusarium oxysporum* population density in the soil at harvest............................................ 122

6.5 Gall rating of sage roots infested with *Meloidogyne incognita* at harvest................................ 123

6.6 Analysis of variance summary of root gall rating response across all treatments for sage at harvest. 123

6.7 Single degree of freedom orthogonal contrasts for summary of gall rating for sage at harvest......... 124

6.8 Population density of *Meloidogyne incognita* in soil of the disease control study with sage at harvest.. 126

6.9 Analysis of variance summary for soil population density of *Meloidogyne incognita* at harvest........ 126

7.1 Analysis of variance summary of plant height data collected over time in the disease control study with lavender........................................ 141

7.2 Analysis of variance summary of the percent dead plant data collected in the disease control study with lavender........................................ 141

7.3 Analysis of variance summary of spike weight and spike length in the first and second harvest of lavender............................................ 147

7.4 *Fusarium oxysporum* infectation of lavender roots in April, 1994................................... 149

7.5 Analysis of variance summary of the effects of *Fusarium oxysporum* infectation of lavender roots at harvest (April, 1994)......................... 149

# LIST OF FIGURES

FIGURE PAGE

3.1 Potted herbs growing in untreated and microwaved field soil........................................ 50

3.2 Effect of inoculum levels of *Fusarium oxysporum* on plant development in the preliminary pathogenicity test with sage...................... 57

3.3 Effect of inoculum levels of *Fusarium oxysporum* on plant development in the preliminary pathogenicity test with lavender.................. 57

4.1 First combined pathogenicity test with sage 7 days after establishment................................ 64

5.1 First combined pathogenicity test with lavender 3 days after establishment.......................... 87

6.1 Diseased sage plants grown at the Green Acres Agronomy farm, Gainesville, Florida............... 108

6.2 General view of the disease control study with sage in August 1992, 4 months after establishment..................................... 108

6.3 Effect of soil amendment on plant height (a) and percent dead plants (b) of sage................... 115

6.4 Effect of mulch treatments on plant height (a) and percent dead plants (b) of sage............... 118

6.5 Effect of mulch treatments on soil temperature at 5-cm depth (a), and at 10-cm depth (b) in the disease control study with sage................... 120

6.6 Sage plants extracted at harvest from a plot treated with lime plus synthetic mulch............ 127

7.1 Diseased lavender plants grown at the Green Acres Agronomy farm, Gainesville, Florida............... 134

7.2 Lime application at establishment of the disease control study with lavender at the Green Acres Agronomy farm, Gainesville, Florida............... 134

7.3 Effect of soil amendment on plant height (a) and percent dead plants (b) with lavender............. 142

7.4 Effect of mulch treatments on plant height (a) and percent dead plants (b) with lavender......... 144

7.5 Effect of mulch treatments on soil temperature at 5-cm depth (a), and at 10-cm depth (b) in the disease control study with lavender............... 146

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# ASSESSMENT AND MANAGEMENT OF THE SOIL-BORNE DISEASE OF SAGE AND LAVENDER IN NORTH-CENTRAL FLORIDA

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A die-back disease was determined to be the most limiting factor in field production of sage (*Salvia officinalis* L.) and lavender (*lavandula* x *hybrida* Balb. ex Ging.) in Florida. The objectives of this research were to: 1) evaluate the pathogenicity of *Fusarium oxysporum* and *Meloidogyne incognita;* 2) determine if there is an interaction between *F. oxysporum* and *M. incognita* race-1; and 3) evaluate the effect of soil management practices on growth and survival of sage and lavender. *Fusarium oxysporum* was pathogenic to both herbs in a preliminary test. A differential host test confirmed the identity of *M. incognita* race-1. Four factorial pathogenicity tests were established with inoculum levels of 0, 100, and 1,000 second-stage juveniles of *M. incognita* race-1 $ml^{-1}$ of soil and 0, 200, and 2,000 propagules of *F. oxysporum* $g^{-1}$ of soil. There was less than 7% mortality of both herbs, even after 9 months. In sage at harvest, *M. incognita* reduced plant height ($P\leq0.05$) and shoot dry weights in one of two tests ($P\leq0.01$). Plant height showed an interaction between pathogens ($P\leq0.05$). *Meloidogyne incognita* reduced plant height ($P\leq0.01$), canopy width, and shoot ($P\leq0.10$) and root dry weights of lavender in one of two tests ($P\leq0.05$). No galls or interaction effect were detected in lavender.

Two disease control studies were conducted using split-plot designs. Main plots were methyl bromide, lime application adjustment of pH to 7.0, and nonamended. Subplots were organic mulch (dry perennial peanut, [*Arachis glabrata* Benth] hay), black synthetic woven mulch, and nonmulched. During the first year, methyl bromide treatment provided a good environment for growth and survival of both herbs ($P\leq0.01$). In sage, organic mulch favored growth while synthetic mulch favored plant survival. Root-knot galls were reduced in plots treated with lime plus organic mulch ($P\leq0.01$). The number of *F. oxysporum* colonies was reduced by synthetic mulch ($P\leq0.10$), mostly in limed plots. In lavender, lime improved survival ($P\leq0.01$), and organic mulch improved plant height ($P\leq0.01$). No root-knot galls were observed in lavender. Lavender is susceptible toinfection by *F. oxysporum*, but resistant to *M. incognita*. Disease control practices for the production of sage and lavender in North Central Florida should include soil fumigation and the addition of lime and organic mulch.

# CHAPTER I

# INTRODUCTION

Public interest in the United States (U.S.) for greater health and diverging ethnic culinary taste has resulted in an increased demand for a broad array of herbs and spices. Simon (1986) estimated that approximately 80% of U.S. herb needs is imported from the Mediterranean region, Africa and Latin America. The 20% produced in the U.S. consists primarily of red pepper (*Capsicum* spp.) and paprika (*C. annum* L.). De Baggio (1990), the International Trade Center (1982), and Tucker (1987) indicated that there is an expanding market for the production of herbs and medicinal plants. The importation of the 17 most important herbs in the USA increased 25% between 1985 and 1987. According to Miller and Harper (1990), this trend has continued with a world market estimated to be 3 billion U.S. dollars in 1993.

Lewis (1984) and Miller and Harper (1990) emphasized that to compete effectively with established herb production centers around the world, producers must be familiar with their production constraints and should incorporate improved and efficient production technologies into their operations. According to Morris and Cracker (1990), an important factor in herb production in the U.S. is the association between organic farming philosophies and herb production practices within gardens and small production areas. In contrast, Kidd (1992) supported efficient and environmentally sound techniques of either organic or inorganic practices as the goal of every production package when more extensive production areas are considered.

Holcomb and Kerr (1987) identified the West Coast, the Midwest, and the Southeast as the most important herb production regions in the U.S. However, little research information is available on herb and spice production. Recent research conducted at the University of Florida indicates that many herbs imported from the Mediterranean, the Far East and the Caribbean regions can be produced in Florida (Angell, 1991) (Moreno, 1990). However, as recognized by Caswell and Preston (1992), some limitations need to be overcome in the introduction of new crops to a region. In the case of herbs, this is especially true for the production of essential oils, for which extensive areas need to be planted under field conditions.

Hasseb and Pandley (1987) concluded that an important constraint in several medicinal and aromatic plants is damage by root-knot nematodes (*Meloidogyne* spp). Root-knot nematodes are the most common and destructive nematodes in Florida. Moreno et al. (1992) found *Meloidogyne incognita* ((Kofoid & White) Chitwood) race 1 to be pathogenic to sage

(*Salvia officinalis* L.) and lavender (*Lavandula* x *hybrida* Balb. ex Ging.).

Through 1989 and 1990, *Fusarium* spp. were isolated at the University of Florida from field-grown plants that appeared stunted with root decay and had black lesions extending up main stems from the soil line. Sage and lavender plants exhibiting these symptoms usually died in the second growing season. Espaillat et al. (1993) obtained 18 isolates of *Fusarium oxysporum* Link from greenhouse plants grown in soil known to be infested. Two of those isolates were suspected of being pathogenic to sage and lavender. Webster (1985) documented that *F. oxysporum*, alone and in combination with *M. incognita*, was capable of reducing plant population and yield of many crop plants. Hence, the hypothesis can be posed that a possible interaction between *F. oxysporum* and *M. incognita* race 1 may be responsible for this disease of sage and lavender.

Palti (1981) emphasized that the control of soilborne diseases can be accomplished at two levels: 1) preplant management of the inoculum through soil treatment with fumigants and pesticides; and 2) restriction of inoculum build-up below potentially damaging levels through the application of soil amendments and alterations of the soil microclimate. Overman and Jones (1986) considered fumigation with methyl bromide an adequate practice to reduce the initial inoculum level of soilborne pathogens in tomato (*Lycopersicon esculentum* L.). Huber and Watson (1970) determined that, in sandy soils, organic mulches raised the "biological buffering capacity," enriching the soils with microflora competitive or antagonistic to pathogens. Also, organic matter provides a source of nutrients and improves water retention, which benefit the crop plant. Engelhard (1989) concluded that raising the soil pH to 7.0 significantly affected the level of Fusarium wilt in *Chrysanthemum* spp.

This research was divided in two major areas. First, the pathogenicity studies under greenhouse conditions included the isolation and identification of the suspected causal agents. Subsequently, the pathogenicity of *F. oxysporum* and *M. incognita*, and the possible interaction among the pathogens on sage and lavender, were studied in four factorial experiments.

The other area of this research consisted of the disease control studies under field conditions. The control studies included evaluations of the effects of the disease on plant development and survival. Also, the influence of cultural practices on pathogen population densities and infection rates was determined. The following different control methods were compared: soil fumigation with methyl bromide, raising the soil pH to 7.0 with dolomite limestone, and applications of organic and synthetic mulches. The goal of the disease control studies was to evaluate alternative, nonchemical, environmental friendly methods of disease management.

Considering the nature of the pathogens suspected to be involved in this die-back disease and the outlined approaches for disease management, the following objectives were established:

I- Evaluate the pathogenicity of the soilborne organisms isolated from diseased plants of sage and lavender;

II- Determine if there is an interaction between *Fusarium oxysporum* and *Meloidogyne incognita* race 1 in causing the die-back disease of sage and lavender;

III- Evaluate the effect of soil management practices on growth and survival of field-grown sage and lavender as influenced by the control of *F. oxysporum* and *M. incognita*.

# CHAPTER II

# LITERATURE REVIEW

## Herbs, Spices, Medicinal Plants and Agronomy

Early humans relied heavily on plants for food, medicine, and much of their clothing and shelter. The botanical skills of these early people should not be underestimated.

> Through most of man's history, botany and medicine were, for all practical purposes, synonymous fields of knowledge, and the shaman, or witch-doctor usually an accomplished botanist represents probably the oldest professional man in the evolution of human culture (M. J. Plotkin, 1991, p. 53).

Anderson (1977) and Rosengarden (1973) documented the history of herbs and spices. One of the oldest medico-botanical descriptions of herbs is compiled in the Papyrus Ebers dating from 1550 B.C. Similarly, the Chinese emperor Shen Nung called the "divine cultivator," wrote a book in 2700 B.C. on medicinal plants and remedies used in his and previous dynasties. As documented by Furnell (1985) and Jones (1956), classical accounts from Greek and Roman literature, as well as the Bible, established the importance of herbs in early history.

Herbs and spices became better known to Europeans after the return of Marco Polo from the Orient in 1298. By the 11th century, herbs were worth their weight in gold and silver. Furnell (1985) and Jones (1956) documented that many towns kept their accounts in pepper (*Piper nigrum* L.), and taxes and rent were determined and paid in pepper. As herbs became highly valued commodities, many Middle East and European empires established trading businesses. They built entire fleets for shipping and accumulated gold and spices. Parry (1969) and Purseglove et al. (1981) considered that world exploration was greatly stimulated by the search for herbs and spices. It was the profitable spice trade that interested the Europeans in sea routes to the Asian tropics where many spices originated. The constant search for a better trade led to the discovery of the American continent in 1492 by Christopher Columbus.

At the end of World War II, the popularity of herbs declined with the advances of modern chemistry, which made possible the massive production of synthetic dyes, medicines, and food additives at cheaper prices. Fuller (1979) claimed herbs maintained their importance for culinary use and home medicine. Recent changes in life styles, progress in the food and pharmaceutical industries, and concern about chemically derived additives and preservatives have increased demand for herbs and other natural products. Landes (1987) considered that survival as well as quality of life depends on the ability of man to exploit plants for treatment of ailments. Progress toward the cure of the serious diseases that afflict humanity depends upon discovery of new chemotherapeutic agents.

Plotkin (1991) considered that the search for new drugs involves the search for extracts or preparations from organisms, in particular higher plants, followed by the purification and characterization of the substance(s) responsible for the desired activity. Once a plant is identified as having potential usage, the ecological and agronomic requirements are determined for field production.

An important consideration in the introduction of a new crop or spice to a region is its ecological amplitudes. Duke and Hurst (1975), using the Holdridge (1966) life zone system, determined the ecological parameters for 200 herbs, spices and medicinal plants. They considered lavender (*Lavandula angustifolia* Mill.) to be adapted to the Cool Temperate Moist Forest life zone, with temperatures of 6º-12ºC and rainfall of 500-1000 mm year$^{-1}$, and to the Warm Temperate Moist Forest life zone, with temperatures of 18º-24ºC and rainfall of 1000-2000 mm year$^{-1}$. Lavender tolerates soil pH from 5.8 to 8.3, but it grows better in alkaline soils. Sage (*Salvia officinalis* L.) was considered to be adapted from the Cool Temperate Moist Forest life zone, with temperatures of 6º-12ºC and rainfall of 500-1000 mm year$^{-1}$, to the Tropical Dry Forest life zone, with temperatures of more than 24ºC and rainfall of 1000-2000 mm year$^{-1}$. Sage tolerates pH from 5.3 to 8.3, but it grows better in alkaline soils. Bryson and Hare (1974) determined the ecological attributes of North Central Florida, placing the Gainesville area in the Subtropical Moist Forest life zone, with temperatures of -8º to 41ºC and rainfall of around 1355 mm year$^{-1}$. Lavender should be better adapted than sage to North Central Florida.

Palevitch (1991) documented that in 1986 and 1987 a search in horticultural abstracts showed that agronomic studies amounted to only 8-9% of the total publications on medicinal plants. These agronomic studies dealt with 79 different plant species. The overall list of medicinal plant publications related to research disciplines other than production techniques comprised more than 300. Simon et al. (1984) compiled an index of scientific literature on herbs, aromatic plants, and medicinal plants of the temperate zone. In the section on diseases they listed 381 references. There were 172 references (45%) dealing with diseases caused by fungi. Ten publications dealt with diagnostics of wilt diseases caused by *Fusarium* spp. on basil (*Ocimum basilicum* L.), fennel (*Foeniculum vulgare* Hill), mint (*Mentha piperita* L.), and coriander (*Coriandrum sativum* L.). There were four reports of disease caused by other fungi on sage (*Salvia* spp.) and none on lavender (*Lavandula* spp.). Reports on disease caused by nematodes were less abundant. Thirty one publications (8%) dealt with nematodes, six of them on *Meloidogyne* spp., one on lavender and none on sage. Diseases caused by fungi were better documented. There were no publications on interactions between pathogens. Viruses of herbs were well documented with 133 publications (35%), two on lavender and none on sage. The residual 20 reports (5%) were on diseases caused by bacteria, of which three were on lavender and none on sage.

Tyler (1986) and Balbaa (1983) considered the domestication of many spices and medicinal plants to be very important for the exploitation of these new crops for their value and also for the preservation of genetic diversity. Franz (1986) believed that domesticated cultivars will provide homogeneous, high quality extracts and will reduce the need for harvesting in the forest, hence avoiding genetic erosion. Improving the performance of herbs and medicinal plants by agronomic techniques can be summarized under the following areas: genetic improvement, optimizing environmental conditions, cultivation under modern cultural practices and post-harvest treatments.

## Biosynthesis of Essential Oils and the Environmental Conditions

Since antiquity, secondary plant products have been used as components of oils and extracts. Simon et al.

(1984) documented that essential oils have been used as ingredients of flavors, soaps, narcotics, perfumes, drugs, and pigments. The major constituents of essential oils are terpenoids. As explained by Goodwin and Mercer (1983), terpenoids are a widespread and diverse group of organic compounds that are derived from a common biosynthetic pathway based on mevalonate; these compounds are synonymously named terpenoids, terpenes or isoprenoids.

Terpenoids are typically found in higher plants, mosses, liverworts, algae and lichens, and some are of insect and microbial origin (Loomis and Croteau, 1980). These compounds are widely distributed in the 94 orders of angiosperms (flowering plants) that are generally acknowledged by taxonomists. They may occur in any part of the plant: roots, heartwood, foliage, inflorescence, or seeds. In nature, they occur in free and combined status.

The most important terpenoids are monoterpenoids. A complete list of their occurrence had been published by Dev et al. (1982). Monoterpenoids are commonly distributed in the essential oils and in the steam volatile and odor constituents of many plant materials. Hence, when studying the chemical composition of essential oils, it is common to look at the profile of monoterpenoids. However, as Banthorpe (1991) pointed out, only 15% of the estimated half-million species of higher plants have been examined for secondary metabolites.

This low percentage of analyzed plants opens a whole avenue for research on plants producing high concentrations of essential oils and other secondary products. Cracker (1990), Farag et al. (1989), and Balandrin et al. (1985) emphasized that, in the last decade, scientists around the world have realize their important activity as anti-bacterial, anti-fungal, and anti-cancer agents.

The fundamental conditions for localization of synthetic sites are structural appearance and a system of cells, tissues, and organs able to accumulate the product. In this respect, Gershenzon and Croteau (1990) claimed that essential oil producing species differ in the place of biosynthesis. Grzunov et al. (1986) and Venkatachalam et al. (1984) studied several species, such as sage (*S. officinalis*), rosemary (*Rosmarinus officinalis* L.), and sweet marjoram (*Oreganum mejorana* L.), in which synthesis and accumulation take place in the leaves.

According to Porter and Shaw (1983) other plants, such as true lavender (*L. angustifolia*), synthesize terpenoids in their leaves and accumulate the compounds in their flowers. Waller and Nowacki (1978) considered castor (*Ricinus communis* L.) an extreme example. Almost all organs of castor are capable of converting nicotinic acid into ricin and accumulate the oil.

Waller and Nowacki (1978) considered that in spite of the fact that essential oil synthesis is gene-governed, environmental conditions play an important role in controlling plant growth and the formation of secondary products. According to Bell and Charlwood (1980) the connection between the formation of primary and secondary products presumes an influence of environmental factors on synthesis and accumulation. These two processes do not separate from the full biomass, but rather represent a productive ratio. They concluded that the production of essential oils may be modified in three manners: 1) influencing the dry matter production, 2) changing the proportion of organs, and 3) modifying the accumulation level.

As explained by Bernáth (1986) and Hornok (1983), the most effective means of increasing synthesis of secondary products of herbs and medicinal plants is by increasing dry matter production. Three models explain the response of different herbs. The linear like equation is the most common one. The response is characterized by the optimum curve, one unit of growth of dry matter will produce one unit accumulation of secondary products. This is the case in reports by Bernáth and Tétényi (1982), in which nutrient and water supply increased overall plant production with no change in the concentration of alkaloid accumulation of poppy (*Papaver somniferum* L.) and mint (*Mentha piperita* L.). The second model response is explained by a power function in which the quantitative accumulation of secondary products is greater than the increase in dry matter production. This is exemplified by increased dry matter of poppy along a sigmoid curve with increased light levels. The last model is the least common and is explained by a orthogonal polynomial function. It consists of reductions in formation of secondary products with increases in dry matter production. This is exemplified by reports by Singh (1977) in which increases in nitrogen levels and irrigation doubled the biomass production of *Matricaria chamomilla* but decreased the volatile oil concentration from 0.64% to 0.59% per unit dry matter.

The accumulation of secondary products will be altered as the number of accumulating organs, the place of synthesis, or the proportion among them changes. Bernáth (1986) concluded that changes in the ratio between underground and above-ground parts influenced the accumulation of secondary products. This ratio seems to be influenced by soil nutrients (N and $P_2O_5$), light intensity (Salisbury and Ross, 1985) and damage to the root system (Tucker and Maciarello, 1990b).

Duke and Hurst (1975) determined the ecological parameters for 200 herbs, spices, and medicinal plants. Piccaglia et al. (1989), Tucker et al. (1984), Hornok (1986) and Franz (1983) documented the alterations the environment and cultural practices produce on growth, yield and composition of the essential oils of sage, lavender, and other medicinal plants.

## Chemotaxonomy of Sage and Lavender

Within the Angiosperms, the family Lamiaceae is the most prolific in the accumulation of monoterpenoids. However, monoterpenoids tend to show more intraspecific and interspecific variations than the other natural products, and chemical races have been detected in individual species of most genera. Essential oil composition differences in plants belonging to the same genus and species are common.

There is evidence that intraspecific, as well as interspecific, variations of monoterpenoids may have an ecological basis. Moreover, intraspecific differences in genetic origin have been hypothesized to arise from biotic selective pressure. Few studies have provided a careful analyses of the sources of variation in relation to environmental influences.

Chemotaxonomy of plants belonging to the same species is based on the presence or absence, as well as the content, of certain terpenoids in the oil (Rhyu, 1979). Basker and Putievsky (1978) stated that the percentage and nature of monoterpenoids in a given plant may vary widely with the season. As an example, Pitarevic et al. (1984) found seasonal variations in the monoterpenoid composition of Dalmatian sage. In a seasonal variation experiment, they found the maximum amount of essential oil in the leaf of Dalmatian sage to be in July. However, they also found the best quality of Dalmatian sage oil to be in plants harvested in October when the content of total thujone is the highest. Thujone is the principal contributor to this spice flavor and aroma.

### Sage Chemotype

Sage (*Salvia* spp.) is a hardy, perennial, profusely branched evergreen shrub of the family Lamiaceae native to the northern shores of the Mediterranean (Malcom, 1979). Sage grows wild on the sunny, rocky, calcareous mountains along the Dalmatian coast of Yugoslavia near the Adriatic sea. Rosengarden (1973) reported the word salvia to come from the latin "salvere," which means to heal or to be of good health. The name refers to the medicinal properties attributed to the herb in antiquity.

Sage has been used since ancient times as a decoction or infusion for spasmolytic, astringent or homeostatic treatments; for wounds; as a diuretic; and to relieve congestions of bronchiolus (Bremness, 1988). It is used in food and perfumery. Also, it is incorporated in many canned meats, sausages, pickles, and liquors as a natural spice possessing antioxidant properties. Malcom (1979) described sage brews called sage ale and sage tea as popular beverages in the 19th century. Piccaglia et al. (1989) considered the most important current commercial uses of sage to be in soaps, candy, meat products, ice cream, chewing gum, baked goods and insect repellents.

The volatile terpenes in the oil are thought to be responsible for sage pharmacological and growth inhibitory activity. Differentiation of sage species is based on the presence or absence, as well as the content, of 10 components in the oil. These include $\alpha$ and $\beta$-pinenes, camphor, 1,8-cineole, $\alpha$ and $\beta$-thujone, limonene, camphene, phellandrene, and bornyl acetate.

Historically, Yugoslavia has been the principal producer of sage oil, followed by Albania. The USDA (1989) calculated that in that year U.S. imports from that region totaled 2,045 kg of sage oil worth over $ 6.8 million. The highest commercial value is given to the Yugoslavian *S. officinalis* (Dalmatian type) oil.

Tucker and Maciarello (1990a) specified that, depending on the genetic and environmental factors, there is great variability in the concentration of the eight principal components of Dalmatian sage: camphene (1.2-8.5%), $\beta$-pinene (0.2-3.4%), $\alpha$-thujone (7.2-47.4%), $\beta$-thujone (1.2-35.6%), camphor (2.7-35.0%), 1,8-cineole (5.5-24.7%), $\alpha$-humulene (1.3-6.9%), and borneol (0.0-15.5%). Most of the commercialized Dalmatian sage is adulterated with Greek sage (*S. fructicosa*), and hybrids among the two are common.

The many cultivars of *S. officinalis* include 'Albiflora,' 'Aurea,' 'Crispa,' 'Holt's,' 'Mammoth,' 'Icterina,' 'Latifolia,' 'Woodcote Farm,' 'Compacta,' 'Berggarten,' 'Purpurascens,' 'Rubriflora,' 'Milleri,' 'Purpurascens,' 'Urbriflora,' 'Salicifolia,' 'Sturnina,' 'Tenuior,' and 'Tricolor.' Burgar et al. (1979) grouped the available essential oil profiles and using the four principal components, developed 13 chemotypes. However, a more practical approach for chemotaxonomy is the grouping by Tucker and Maciarello (1990a). Known cultivars were classified in five chemotype groups depending on the order of concentration of the four most important oil components for flavor and aroma. The five chemotaxonomy groups are the following: 1) includes the cultivars Berggarten and Icterina with camphor > α-thujone > 1,8-cineole > β-thujone; 2) includes Tricolor and Woodcote Farm with camphor > α-thujone > β-thujone > 1,8-cineole; 3) includes the cultivar Albiflora with β-thujone > camphor > 1,8-cineole > α-thujone; 4) includes the cultivar Compacta with 1,8-cineole > camphor > α-thujone > β-thujone; and 5) includes the cultivars Purpurascens and Rubriflora with α-thujone > camphor > β-thujone > 1,8-cineole.

At the University of Florida, a cultivar used for the last 4 years in research dealing with different aspects of herb production has been placed in chemotype 2 (Tucker, unpublished) (Appendix A-1). The cultivar has an oil profile acceptable on the market. The cultivars Tricolor and Woodcote Farm belong to this group and are produced for fresh and essential oil markets in California. Although they do not compete well with the Dalmatian sage from Yugoslavia, the unstable political situation in that region has limited production and, consequently, an increase in demand for sage oil produced in California.

### Lavender Chemotype

Lavender (*Lavandula* spp.) is a perennial shrub which belongs to the family Lamiaceae and is native to the south coast of France. Morton (1976) considered the most important species in the genus to be *L. latifolia* Med., called spike lavender; *L. angustifolia*, called true lavender; and *Lavandula x intermedia* Emeric ex Loisel., called lavandin, which is a sterile hybrid between the first two species.

Morton (1976) stated that the words lavandula and lavender come from the Latin "lavare," which means to wash. Tucker (1985) considered the origin of the word a reflection of the herb's fragrant and clean aroma and its use as a bath oil. Morton (1976) documented that in old times, lavender flowers were candied and eaten to relieve upset stomachs. Today true lavender oil is used in perfumes, bath oils, gargles and body oils. Spike and lavandin oils are used primarily for soap and industrial food flavorings (Marutani et al., 1987, and Zaika et al., 1983).

The U.S.D.A. (1989) reported that the U.S.A. imported in that year 277 Mg of undifferentiated lavender oil with a value of $ 4.21 million. As specified by Morton (1976) and Lammerink et al. (1989), lavender should be harvested when the plants are 70% to full bloom to obtain maximum oil yield. By harvest time, the flowers of lavandin plants can be 30 cm long.

Lavandin oil does not possess the high quality of the true lavender oil, but has a much higher yield potential and consequently a better production cost efficiency than true lavender. Tucker and De Baggio (1984) considered lavandin cultivars more attractive as commercial crops. There are several cultivars of lavandin, including 'Albrialis,' 'Super' and 'Crosso.' 'Crosso' is the predominant lavandin cultivar in France; it has great vigor, productivity, and resistance to yellow decline caused by a mycoplasma-like organism. In 1983, 'Crosso' contributed 70% of the total French lavandin oil production, compared with 20% for 'Albriolis' and less than 10% for 'Super' (Lalande, 1984).

The cultivar Crosso was introduced to Florida four years ago. Twelve plants were placed under greenhouse conditions to observe their development. Preliminary observations indicated that it will not do well in the Florida climate. Seven plants died in the heat of summer and flowering was limited to few or no flowers (unpublished).

Lammerink et al. (1989) characterized lavender oil by physico-chemical properties and percentages of 29 components. Other characterizations have been made in France and the U.S.A. The principal criterion for quality in lavandin oil is its resemblance to true lavender oil. Lalande (1984) specified that high quality lavandin oil should contain relatively low levels of 1,8-cineole, camphor, and borneol, but relatively high levels of linalyl acetate, terpinen-4-ol, and lavandulyl acetate. The objective of breeding programs for lavandin has been to improve the quality of interspecific hybrids by reducing the content of undesirable components to the minimum level. The Association Francaise de Normalization provided specifications for percentages of 10 components in commercial 'Albrialis' and 'Grosso' oils. These specifications are used as a guide for commercial acceptability of the oil and to classify the different chemotypes.

Prager and Miskiewicz (1979) documented a system used to distinguish oil quality and establish price of oil, in traditional producing regions of France. While it is impossible to mistake true lavender for spike, and vice-versa, the problem is to distinguish between lavender and lavandin. However, camphor has a determining significance due to its presence or absence characteristic. True lavender oil does not contain camphor, except for traces, which can not be detected. All spike cultivars and known hybrids contain camphor. This is important because the lower the camphor content in lavandin, the closer the hybrid appears to be to lavender through all its other characters. So, a camphor-free lavandin oil could be sold as true lavender oil at higher prices.

The chemotype used in this research was identified by Tucker et al. (1993) as *Lavandula* x *hybrida* Balb. ex Ging. This hybrid is commonly sold as *L* x heterophylla or *L. latifolia* in the U.S. (Appendix A-2). The oil composition is dominated by 1,8-cineole (33.5±1.7%) and camphor (12.4±0.4%) with an oil yield of 0.64±0.09%. This hybrid is considered of commercial importance in the essential oil market.

## Diseases Caused by Soilborne Fungi in Herbs

Soilborne fungal pathogens are often localized or restricted to a particular part of the root, such as the vascular system, cortex, or tips. Gardner et al. (1985) found the most negative effect on the host to be the interference with the water and solute movement from the roots to the foliage. Bruehl (1987b) found occlusion of the vessels by gels and tyloses formation to be one of the mechanisms by which die-back diseases impair water movement.

Agrios (1988) considered occlusions important, because they increase the viscosity of the xylem stream. Bald (1969) claimed that if the occlusions occur promptly, it is beneficial because rapid gel formation followed by tyloses may localize the pathogen in the roots so it does not become systemic. Garrett (1970) concluded that a pathogenic fungus or race will produce a slow plant host response, allowing microconidia to proceed from vessel to vessel in the root. Beckman and Talboys (1981) and Campbell and Madden (1990) determined that in these cases vascular occlusions occur, but only after extensive host invasion and productivity is already affected. Timing and vigor of the host response determines whether the plant is susceptible or resistant.

Herbs of Mediterranean origin have been found to be susceptible to wilts and die-backs. Raabe (1989) reported that damping-off and sudden die-backs could be caused by a number of fungi, but that the principal pathogens involved are *Pythium* spp., *Rhizoctonia solani* Kuhn. and *Fusarium* spp. *Rhizoctonia solani* has been reported on dill (*Anethum graveolens* L.), camomille, rosemary, mint, and thyme (*Thymus vulgaris* L.). No reports were found of diseases caused by *Pythium* spp. on herbs.

Millikan (1990) found die-back on herbs to be caused by infestations of species of *Fusarium*, *Rhizoctonia* and *Phytophthora*. Wheeler and Boyle (1971) isolated *Phytophthora parasitica* Dast. from diseased rosemary plants grown in Arizona and California. As stated by Tucker and Maciarello (1990b) and Farr (1989), diseases caused by soilborne pathogens not only can shorten the production cycle, but also reduce the essential oil quantity and quality in herbs and medicinal plants.

Purseglove et al. (1981) documented that spices of tropical origin also have been found susceptible to wilts and die-backs. Cook (1975) reported macadamia (*Macadamia integrifolia* Mueller) to be susceptible to Phytophthora canker caused by *Phytophthora cinnamomi* Rands. Thurston (1989), Purseglove (1991), Weber (1973) and Holliday (1980) considered crown rot caused by either *Phytophthora parasitica* or *P. palmivora* to be the most important disease of black pepper (*Piper nigrum* L.). Purseglove et al. (1981) reported a root rot disease of vanilla (*Vanilla planifolia* A.) in Puerto Rico caused by *Fusarium batitis* Wollenw f. sp. vanillae. Saleem and Klan (1985) indicated that root and stem rot of sesame (*Sesamum indicum*), caused by *Macrophomina phaseolina* (Tassi) Goid., is an important disease in Pakistan.

In traditional clove (*Syzygium aromaticum* (L.) Merr. & Perr) production areas, the most serious problems are sudden death, caused by *Valsa eugeniae*, and die-back, caused by *Cryptosporella eugeniae* (León, 1968; Purseglove, 1991). Cook (1978) reported bacterial wilt caused by *Pseudomonas solanacearum* (Smith) as a serious problem of ginger

(*Zingiber officinale* Rosc.) in Hawaii and Australia. Also, Fusarium wilt caused by *Fusarium oxysporum f. zingiberi* (E.F. Smith) was reported as a severe disease in Australia, India, and Hawaii. The Ministry of Agriculture and Fisheries of Jamaica (1976) listed a root spot of ginger caused by *F. oxysporum*.

Harper (1975) reported black root disease of tea (*Camellia sinensis* (L.) O. Kuntz), caused by *Rosellinia* spp., was a major problem in the highlands of Indonesia, while red root disease, caused by *Ganoderma* spp., is a major problem in the lowlands. Wellman (1974) listed the following controls for most of these diseases in the tropics: cultural practices, such as planting healthy plant material; chemical treatment of plant materials; long rotations with nonhost crops or fallow; avoidance of mechanical or insect damage; and sanitation.

Tucker and Maciarello (1990b) reported lavender and lavandin were infected by *Coniothyrium lavandulae* Marie, *Phytophthora* spp. and *Fusarium solani* (Mart.) in Delaware, USA. Before that, Humphrey-Jones (1981) diagnosed *Coniothyrium lavandulae* on *L. angustifolia*. The Ministry of Agriculture of England (1980) reported a scab disease caused by *Phoma lavandulae* Gabotto. Young shoots turn yellow and wilt, later the branches die-back and the disease spreads, and the plant subsequently dies. They recommended the removal and burning of infected plants. Lammerink et al.

(1989) reported the susceptibility of sage 'Abrialis' to yellow decline caused by a mycoplasma-like organism. The cultivar Crosso is resistant to yellow decline and has replaced 'Abrialis' as the major producer of lavandin oil.

Rick (1943) reported root-rot problems in sage. Tucker and Maciarello (1990b) reported sage to be susceptible to species of *Phytophyhora*, *Verticillium*, and *Fusarium*. In the same study, rosemary was found to be attacked by *Verticillium* spp. Also, basil (*Ocimum basilicum* L.), sweet marjoram, and dill were found to be susceptible to *Pythium* spp. The Ministry of Agriculture of England (1980) reported *Verticillium* wilt, caused by *V. dahliae* Kleb, to be a major disease of sage, but the disease has been overcome largely by propagation from clean stock.

## Disease Caused by *Fusarium oxysporum* in Sage and Lavender

As stated by De Baggio (1987), sage grows in soils with pH ranging from 4.9 to 8.2. Morton (1976) suggested that sage can grow well in most temperate climates, but that it does poorly in most tropical environments. Tucker and Maciarello (1990b) discovered that, under hot and humid conditions, sage becomes susceptible to soilborne fungi.

As with sage, lavender should be planted in sunny, wind-sheltered areas with well drained soils. Fuller (1979) and Tucker and Maciarello (1990b) determined the optimum soil pH to be around 6.0. Bremness (1988) recommended avoidance of over-watering because it may lead to disease problems. Researchers such as Upadhyay and Bordoloi (1975), Raabe (1989), Millikan (1990) and Tucker and Maciarello (1990b) reported sudden die-back and damping-off caused by soilborne fungi as problems of lavender and other herbs in hot humid environments. Searches of the published literature conducted by the author and by Simon et al. (1984) failed to find a report of a disease caused by *F. oxysporum* on sage and lavender.

## Disease Caused by *Meloidogyne* spp. on Herbs

Root-knot nematodes (*Meloidogyne* spp.) are the most damaging plant-parasitic nematodes in the world. As identified by Chitwood (1949), the most common root-knot species are *M. arenaria* (Neal) Chitwood, *M. incognita* (Kofoid & White) Chitwood, *M. hapla* Chitwood, and *M. javanica* (Treub) Chitwood. Sasser (1980) considered that these species constitute about 98% of the root-knot nematode populations found throughout the world. Jepson (1987) and Sasser and Carter (1982) mentioned the existence of more than 50 species of root-knot nematodes and various races of some species.

As explained by Dropkin (1980) and Taylor and Sasser (1978), races within plant-parasitic nematode species are recognized as morphologically similar, but with different host preferences. Races occur in some species of the genus

*Meloidogyne*. The method most often used to separate races of *Meloidogyne* spp. is a differential host test, such as the that described by Taylor and Sasser (1978). This differential host test uses differential host responses to distinguish among *M. arenaria* races 1 and 2 and *M. incognita* races 1, 2, 3 and 4. The tomato (*Licopersicon esculentum* L.) 'Rutgers' is used as the indicator plant because of its susceptibility to all *Meloidogyne* spp. and races. Cotton (*Gossypium hirsutum* L.) 'Deltapine 16,' tobacco (*Nicotiana tabacum* L.) 'NC 95,' pepper (*Capsicum frutescens* L.) 'California Wonder,' watermelon (*Citrulus vulgaris* Thunb.) 'Charleston Gray,' and peanut (*Arachis hypogea* L.) 'Florunner' are normally included as the test plant species. Jepson (1987) explained that in a differential host test all plants are inoculated with the root-knot nematode to be identified. Galls will form in tomato and in one or more of the test plant species. The test plant with galls is considered a differential host plant.

Crop and yield losses caused by root-knot nematodes have been calculated to be at least 5% annually in most crops (Sasser and Carter, 1982). Losses of up to 60% have been reported in certain crops under field conditions. Several scientists have studied the mechanism of root damage by *Meloidogyne* spp. Ayoub (1980) and Franklin (1979) found that the damage starts when second-stage juveniles (J2) establish a feeding site in the vascular cylinder and the females become sedentary. Tender cells in the pericycle and vascular cylinder are perforated with the stylet, and secretions from the esophageal glands are injected into the cells. Then, as explained by Bird (1974) and by Khan and Esfahani (1992), cell enlargement in the vascular cylinder is initiated, forming giant cells. Galls consist of giant cells formed by hypertrophy and hyperplasia.

Sasser and Carter (1982) stressed that *Meloidogyne* spp. can attack almost all cultivated crops, and spices of temperate and tropical origin are no exceptions. Luc et al. (1990) claimed that almost all tropical spices, such as vanilla, clove, nutmeg (*Myristica fragrans* Houtt), cinnamon (*Cinnamomum zeylancium* Ness), cumin (*Cuminum cyminum* L.), fenugreek (*Trigonella foenum-graecum*), coriander (*Coriandrum sativum* L.), black pepper, ginger, and cardamom (*Elettaria cardamomum* Maton), are hosts of *Meloidogyne* spp. Harper (1975) documented *M. incognita* to be a serious problem of tea nurseries in Indonesia. Vilsoni (1979) found heavy infestations of *M. incognita* and *M. javanica* in ginger rhizomes, with 57% yield reduction. Also, Colbran (1962) found ginger rhizomes to be smaller, deformed, and discolored in fields heavily infested with root-knot nematodes.

Other studies have shown other herbs with varied levels of resistance and susceptibility to root-knot nematodes. Ibrahim et al. (1983) found fennel (*Foeniculum vulgare* Hill) to be resistant to *M. incognita* race 3, *M. arenaria* race 1 and *M. javanica*. Anise (*Pimpinella anisum* L.), celery (*Apium graveolens* L.), caraway (*Carum carvi* L.), coriander, dill and parsley (*Petroselinum sativum* Hoffm.) were found to be either moderately susceptible or susceptible to these nematodes. Ali and Koshy (1982) reported susceptibility of cardamom to *M. incognita* and *M. javanica*. Shah and Patel (1976) found cumim yield to be reduced by *M. incognita*. Radewald et al. (1972) reported parsley to be susceptible to *M. incognita*. Hasseb and Pandey (1989) found Japanese mint (*Mentha arvensis* L.) to be attacked severely by *M. incognita* and *M. javanica*.

Some herbs have proven utility in the control of root-knot nematodes. Sukul et al. (1974) reported that aqueous extracts of ginger, garlic and chili pepper (*Capsicum annum*) reduced galling and population levels of *M. incognita*. Hasseb and Pandey (1987) found basil to be host of *M. incognita* and *M. javanica*. In Florida, Rhoades (1988) reported basil to be highly susceptible to *M. incognita* in greenhouse studies. Research conducted by Murthy and Elias (1967) reported only light infection of *M. incognita* in basil with small galls. Later, Hasseb and Pandley (1989) documented that oil cakes from herbs such as basil had shown nematicidal properties. Furthermore, Chatterjee et al. (1982) reported reductions in *M. incognita* populations when exposed to the crude essential oil of basil, of which the major constituent is the terpenoid linalool. Intraspecific variations between species and chemotypes of basil may count for these contradictory results.

## *Meloidogyne* spp. on Sage and Lavender

Hasseb and Pandley (1987) and Miller (1977) concluded that root-knot nematodes may be an important constraint in the production of several medicinal and aromatic plants. Lisetskaya (1973) found *M. hapla* and *Paratylenchus hamatus* in roots of sage. Also, he observed that *Meloidogyne hapla* caused moderate to severe growth reduction of lavender.

In Florida, there have been some earlier studies on the pathogenicity of nematodes on herbs. Nematode screening was conducted with sage and other herbs by Chitwood (1949) using *M. arenaria* race 1, *M. incognita* races 1 and 2, and *M. javanica*. In this study, sage developed galls in response to inoculations with all *Meloidogyne* spp. and races tested, while other herbs showed fewer galls or no galls.

Moreno et al. (1992) found *Meloidogyne incognita* race 1 to be pathogenic to sage (*Salvia officinalis*) and lavender (*Lavandula* x *hybrida* Balb. ex Ging.). On sage and lavender, *M. incognita* race 1 produced moderate levels of galls and egg masses. These results demonstrated the importance of continued research in this area. In sage, germplasm screening needs to be explored so that a resistant cultivar can be identified or developed.

## Interaction between *Fusarium* spp. and *Meloidogyne* spp.

The association of *Meloidogyne* spp. with secondary pathogens poses a potential threat to healthy crops. Atkinson (1892) was the first to report a nematode-fungus complex. He observed that *Fusarium* wilt in cotton was always severe in fields infested by root-knot nematodes. Since this report, many researchers, such as Porter and Powell (1967), Bergerson (1975) and Montalvo and Melendez (1986), have extensively studied the disease complexes involving *Meloidogyne* spp. and *Fusarium* spp. on several crops. Moreover, Sikora and Carter (1987) suggested that the reports of many important interactions are contradictory or at least cannot be verified.

Powell (1982) and Taylor and Sasser (1978) documented interactions of *Meloidogyne* spp. with species of *Aspergillus*, *Fusarium*, *Helminthosporium*, *Phytophthora*, *Pseudomonas*, *Pythium* and *Verticillium*. In most of these disease complexes the nematode was considered the primary pathogen, and the other organisms were considered the secondary pathogens. The amount of disease caused by the secondary pathogen depends on the effect of the primary pathogens.

In Puerto Rico, Montalvo and Melendez (1986) and Varela (1983) reported that the severity of *Fusarium* wilt of yam (*Dioscorea rotundata* Poir) and *Fusarium* root rot of "ají" (*Capsicum annuum*) were increased by *M. incognita*. Negrón and Acosta (1989) found an interaction between *F. oxysporum* f.sp. *coffeae* and *M. incognita* in causing *Fusarium* wilt of coffee in Puerto Rico. Webster (1985) documented the interaction of *F. oxysporum* with *M. incognita* as capable of reducing plant populations and yield of many crops in Florida. Lacy et al. (no date) provided the only documentation of the effect of disease complexes on herbs and spices; in these study nematodes were found to increase the severity of *Verticillium* wilt in mint.

The association of *Meloidogyne* spp. with other pathogens appears to weaken plant resistance to specific soilborne diseases. Diomande et al. (1981) documented such a case in peanut (*Arachis hypogaea* L.) 'NC 3033,' in which resistance to *Cylindrocladium* black rot is lost in the presence of *M. arenaria* race 2. Dropkin (1980) observed the loss of resistance to *F. oxysporum* with the presence of *Meloidogyne* spp. in cotton (*Gossypium hirsutum*). Powell (1971) found that nematodes usually do less well when the root system suffers from another soilborne pathogen infection. Bruehl (1987b) corroborated Powell's (1971) claim by pointing out that the organism accompanying the nematode may gain, but not the nematode.

## Soil Management Practices for Soilborne Disease Control

Disease management is a process which begins with accurate diagnosis of the inducing or causal agents of disease. Grogan (1981) concluded the most important question to answer in disease diagnosis is whether an infectious agent is involved, and, if so, to what extent. If the cause of the disease is not known, the most efficient and effective control measures can not be taken (Shokes et al., 1987). Cowling and Horsfall (1979) praised Koch's (1891) procedures to prove that one contagious disease is caused by a suspected pathogen. The technique is known as Koch's postulates and includes the isolation, identification, demonstration of pathogenicity of the suspected pathogen, reisolation of the pathogen and identification of the pathogen.

Shokes et al. (1987) explained that disease assessment may be performed to evaluate cultivar resistance, to compare cultural control measures, to compare the efficacy of pesticides, to predict future disease, or to project the loss of potential yield. In any disease assessment procedure, the method of sampling must be considered. Campbell and Noe (1985) suggested that the amount of disease on plant roots may be estimated at one particular time in the history of the crop, usually at harvest.

Berger (1980) recommended that it would be more advantageous to follow the progress of root diseases over time without destructive sampling. However, this is difficult, and usually experiments are designed with excessive experimental units to permit estimates of pathogen population(s) with sampling at several intervals. Correll (1992) considered the attempts to detect or isolate a specific soilborne fungus a frustrating task if the wrong plant tissue is sampled or the wrong time of sampling is selected. Noe et al. (1991) concluded that high coefficient of variation values are common to pathogenic evaluations of soilborne pathogens.

Latin (1986) noted that there is a scarcity of specific information regarding herb disease control. Very few disease-resistant varieties have been identified and none of the common broad-spectrum fungicides are registered for use on herbs. The pending loss of methyl bromide is necessitating the development of alternative methods of control of soilborne fungi and nematodes. Although there are other chemical methods for the control of these pests, none of them provide such a broad-spectrum control of soilborne pathogens.

Fungicides such as benomyl, metalaxyl and Captan® have been recommended for the control of species of *Rhizoctonia*, *Pythium*, *Fusarium* and many other pathogens. However, these and many other fungicides are not registered for use on culinary herbs due to residues left from initial applications and the organic philosophy tied to herb production. Also, the issues of environmental impact and food chain contaminations put other pesticides in continuous recertification, with the chance of them being taken off the market. Thus, there is an urgent need to look to cultural controls, such as mulches, crop rotation, liming, soil aeration, sun exposure, soil drainage and host resistance, as means of controlling soilborne diseases.

There are differences between the optimum environmental soil condition for growth of plants and for the development of a disease complex or a single pathogen. Palti (1981) pointed out that the development of a healthy crop depends on the suitability of a soil for adequate root growth. Any practice which improves texture, composition and reaction of the soil will have an impact on root development and may increase the capacity of the plant to overcome disease. Baker and Snyder (1965) and Malajczuk (1983) considered that such a practice will reduce pathogen survival and pathogenicity. Several cultural practices provide these conditions. As Zentmyer and Bald (1977) stated, interactions among the physical, chemical, and biotic environment of the soil and the plant are used for the control of soilborne pathogens. Kharband and Tewari (1996) proposed that disease control by environmental changes may act through its effect on other organisms of the micro-biota, which could result in biocontrol or protect the crop.

## Cultural Practices

Palti (1981) emphasized that the role of cultural practices in the protection of crops from disease includes the prevention of the introduction of inoculum into the region, and the elimination of additional and alternate hosts of potential pathogens. In high investment and modern agricultural systems the risk of pest damage needs to be minimal. The use of cultural practices as substitutes for soil fumigation or any other method for the control of soilborne pathogens presents a dilemma. There are different opinions as to whether or not a single cultural practice could provide the required protection and security to farmers and investors. Trivedi and Barker (1986) and Scherf and Macnab (1986) stressed the need for an integrated approach with the use, if possible, of resistant varieties, bio-control, fallow, sanitation, soil amendments, and intercropping systems.

Crooley et al. (1989) studied agricultural ecosystems and claimed they support a large and varied soil fauna, whose importance is only beginning to be recognized. Stoll (1986) stated that in natural forests, the importance of faunal activities are well recognized. Faunal activity influences decomposition systems, rates of nutrient regeneration, and soil structure. However, fauna in agricultural systems are largely ignored by agronomists, except for those animals causing economic damage to crops.

The importance of the large and varied fauna of nematodes, mites, collembolans, and enchytraeids, which occur in undisturbed soils, needs to be taken more into consideration. Today, there is little specific information on faunal impacts that might be used to develop efficient techniques for managing heavily cultivated agricultural fields.

A rich flora and fauna of soil microbiota may reduce the danger of epidemic outbreaks of disease caused by soilborne pathogens (Wood, 1989). Wilhelm (1965) and Malajczuk (1983) stated that the biological complexity of healthy soils assures associative, competitive, and antagonistic relations. These characteristics limit population explosions and bring about balance. Cook (1977) and Hoitink and Hadar (1992) claimed that abundant fauna and flora maintain and even enhance soil fertility, and thus high productivity. Microarthropods which occupy all trophic levels within the detritivore food web, for example, may offer the advantage of a controlled, continuous release of minerals.

Cook and Baker (1983) considered biocontrol a promising technique in integrated control of soilborne pathogens, because in most of the cases biocontrol agents are pathogen specific. Traditional farmers have used biological control through their development of suppressive soils and the use of antagonistic plants. Thurston (1992) reported that the oldest example of suppressive soils is the addition of large quantities of organic matter by Chinese farmers; pathogens are killed by the heat or the amount of organic matter in composting processes or by microbial interactions in soil amended with organic matter.

Most of the commonly cultivated herbs, originated in the neutral or alkaline soils of the Mediterranean Region, western and southern Europe, Asia Minor, and northern Africa. Barker (1986) considered that optimum growth for most herbs occurs at pH 6 to 7. Duke and Hurst (1975) determined lavender will not grow at pH lower than 5.8 and labeled it a very sensitive plant. Sage was considered a less sensitive plant because it tolerates a pH of 5.3.

There is no doubt of the significance of pH in herb production, yet we do not understand how soil pH affects many pathogen-host interactions. Toussoun (1975) listed Fusarium wilt of cotton (race 1) and club root of cabbage as favored by acidity. Englehard and Wolz (1973) found that complete control of Fusarium wilt of Chrysanthemums (*Chrysanthemum* x *morifolium*) can be obtained by growing plants in pots in a regime of high lime, nitrate fertilizer, plus benomyl®. They found each factor to be additive in increasing increments of control. Bockus and Claasen (1985) reported that liming in acid soil in Kansas reduced the incidence of *Cephalosporium* stripe in the field. Bruehl (1987a) found it was not often practical to control diseases by modifying soil pH alone, even though this practice is effective for control of some diseases.

Pearson and Adams (1967) calculated most liming applications to be at rates of 2.5 to 15 metric tons $ha^{-1}$ $yr^{-1}$. But to prevent overliming, recommendations usually are limited to 10 metric tons $ha^{-1}$ $yr^{-1}$. The need for reliming is determined by soil texture, leaching, cropping, cultivation practices, fertilization, and the type and fineness of the original liming material. Robbins (1988) reported that sage growth and yield were better when soil was limed with 13 to 17 metric tons $ha^{-1}$ $yr^{-1}$ of dolomitic limestone.

Antagonistic or trap plants used in biocontrol of soil-borne pathogens contain chemical compounds which are toxic to pathogens. Giebel (1974), Pi and Rhode (1967), Veech (1981), Khan et al. (1971) and Menton (1977) have studied the effects of antagonistic plants on nematodes. Hackney and Dickerson (1975), Dominguez et al. (1986) and Good et al. (1973) have studied marigold (*Targetes minuta* L.), beggarweed (*Desmodium tortuosum* L.), hairy indigo (*Indigofera hirsuta* L.), neem (*Azadirachta indica* A. Juss) and other plants for use as suppressive crops for root-knot nematode and fungal control. Varma et al. (1978) found sesame (*Sesamum indicum* L.) and marigolds to be toxic or repellant to *M. incognita* and *M. javanica* in India. Trivedi and Barker (1986) found biochemical and histological changes to be involved in plant root resistance, including the production of organic acids and polyphenols. Wallace (1973) concluded that in most cases, the resistance reaction takes place after root penetration.

Resistant plants should cause a reduction of nematodes and fungi. Cook (1974) considered the reduction of the size of root-knot females to be an expression of resistance. Franklin (1979) contemplated that if a plant allows a pathogen to feed and reproduce, the plant should be considered a susceptible host. A tolerant plant may or may not affect the reproduction of the pathogens. The degree of tolerance is often measured by a yield component, such as dry matter production. Determination of plant infectation and pathogen population density in the soil are used to determine whether a plant is susceptible or resistant to a particular soilborne pathogen.

## The Use of Organic Mulches

Organic amendments and mulching have and will keep playing a major role in integrated control of soilborne diseases. Also, reduction in soilborne pathogens, especially fungi and nematodes (Rich, 1986), and other important agronomic benefits are advantages of mulches and amendments. Thurston (1992) warned that care must be taken when choosing the organic material to be used, avoiding liberation of phytotoxic products or the introduction of a new pathogen. Pathogen dissemination is not a problem in the proper production of compost because the generated heat kills them. The major problem with amendments and mulches is that they are needed in large quantities.

Mulching has different meanings to different people. Wilson and Akapa (1983) defined a mulch as any covering placed over the soil surface to modify soil physical properties, create favorable environment for root development and nutrient uptake, and reduce erosion and degradation. Some of the advantages of organic mulches impact plant development directly, others improve or prevent soil deterioration. Gallaher (1980) proposed that mulch materials could be residues from the current or recent crop, such as those from minimum tillage, multiple cropping systems. Also, mulches could be brought from other places. Fresh and dry plant material and compost can be applied as soil amendments before and after crop planting. The use of synthetic mulches has become very popular in the production of high value crops, such as vegetables and small fruits. The practice typically includes soil fumigation and soil coverage with polyethylene film.

As a protective cover on the soil surface, mulches prevent the deterioration of soil structure that may be caused by heavy rain. This is important because it can be used to reduce soil erosion at the surface, and to increase infiltration of water into the soil. Gregory et al. (1959) considered organic and synthetic mulches important in reducing splash dispersal of fungi and other soilborne pathogens.

Mulches act as insulators of the surface against rapid changes in temperature. Partially covered soils tend to increase in temperature differentially than bare soil; increased temperature may be due to the color of the residue and reflection of the incident sun rays, or in part due to moisture changes. Covered soil can also retain latent heat. Tucker and Maciarello (1990b) found dark colored organic mulches, such as wood chips, shredded bark or rotted sawdust, promoted wilt fungi. On the other hand, light-colored inorganic mulches, such as sand and gravel, increased lavandin flowering and survival.

Properly utilized mulch can result in significant increases in yields of crops. Organic mulches are a good source of organic matter and mineral nutrients in soil. Upon decomposition, mineral elements contained in the mulch are released and become available to the plant. At the beginning not all of the mineral elements are released. The quantity released at early stages of decomposition are related to 1) the percentage of the nutrient in the residue and 2) the susceptibility of the residue to decomposition.

Plant parasitic nematodes cause economically significant crop losses in tropical, subtropical, and temperate climates. They act as pathogens, predisposition agents, and virus vectors. Noe et al. (1991) claimed that incorporated organic mulches have shown evidence of suppressive effects on plant-parasitic nematodes. An extensive literature available on this subject was compiled by Muller and Gooch in 1982; it documented the use of several organic amendments around the world for management of nematodes.

How mulches and organic amendments control plant nematodes has been given several explanations, depending on the mode of action of the material used and the nematode under control. Fish residues and other animal waste material provide interesting examples of soil amendments used for the control of plant-parasite nematodes. Rodríguez-Kábana et al. (1989) found that when chitinous material, derived from blue crab (*Callinectes sapidus*), was incorporated in the soil at proportions of 1:1, there was an increased parasitism of eggs of root-knot nematodes (*Meloidogyn*e spp.) by fungi.

The short-term effects of organic mulches on soilborne plant pathogens may be related to changes in the environmental parameters, such as temperature and moisture (Huber and Watson, 1970). As the decomposition process progresses toward the chemical and biological balance of the soil, the trophic levels will be slowly reestablished to normal levels. Also, the processes of N fixation, nitrification, and mineralization of N and P will be reestablished.

In the long term, the application of organic mulches will result in an increase of microbial and protozoan activity. Aust et al. (1980) determined an increase of saprovore, bacterivore, and predatory nematodes, while plant parasites were decreased. Also, predatory mites and collembola increased, having a negative effect on plant parasitic nematode populations.

The long term incorporation of mulches contributes to the development of long term, sustainable cropping systems. Although, there is the possibility of phytotoxicity by products from decomposition and plant pathogens or other pests may be harbored in mulches, the positive benefits mentioned above generally have been found to greatly outweigh potential negative aspects.

The cultural practices discussed above have supplied human needs for thousands of years. The potential of cultural practices in integrated control of soilborne diseases is now getting recognition among scientists. These are promising areas of research, especially in developing countries where the cost of nematicides and fungicides is prohibitive. However, Doran and Werner (1990) clarified that the importance of a given management system in the soil environment is expected to vary with soil, climate, and cropping history. Any information generated concerning the management of crop disease by alterations of the soil environment will have to refer to specific conditions and specific host-pathogen combinations. Cultural practices constitute long-term investments, because results become apparent gradually and are not usually as incisive or dramatic as those of some fungicide treatments. As a result, the assessment of cost-benefit relations is complicated.

# CHAPTER III

# ASSESSMENT OF PATHOGENS IDENTITY AND PATHOGENICITY

## Introduction

Herbs of Mediterranean origin have been found to be susceptible to wilts and die-backs. Raabe (1989) reported that damping-off and sudden die-back could be caused by a number of fungi, but that the principal pathogens involved are *Pythium* spp., *Rhizoctonia solani* Kuhn., and *Fusarium* spp. Millikan (1990) found die-backs on herbs to be caused by infestations of species *Fusarium*, *Rhizoctonia*, and *Phytophthora*. Tucker and Maciarello (1990b) and Farr (1989) stated that diseases caused by soilborne pathogens not only can shorten the production cycle, but also reduce the essential oil quantity and quality in herbs and medicinal plants.

Through 1989 and 1990, *Fusarium* spp. were isolated from field grown sage (*Salvia officinalis* L.) and lavender (*Lavandula* x *hybrida* Balb. ex Ging) plants at the University of Florida. The plants appeared stunted and had root decay and black lesions extending up the main stem from the soil line. Tucker and Maciarello (1990b) reported that lavender and lavandin were infected by *Coniothyrium lavandulae* Marie, *Phytophthora* spp. and *Fusarium solani* (Mart.) in Delaware. The same authors, found sage to be susceptible to species of *Phytophyhora*, *Verticillium* and *Fusarium*. Rick (1943) documented root-rot problems in sage. A search of current published studies and a literature review by Simon et al. (1984) failed to obtain reports of disease caused by *Fusarium oxysporum* Link on sage and lavender.

Hasseb and Pandley (1987) concluded that root-knot nematodes (*Meloidogyne* spp.) are an important constraint in the production of several medicinal and aromatic plants. Lisetskaya (1973) diagnosed *Meloidogyne hapla* (Chitwood) in roots of sage and lavender. Chitwood (1949), in a nematode screening test, found sage to be susceptible to *M. arenaria* (Neal) race 1, *M. incognita* (Kofoid and White) races 1 and 2, and *M. javanica* (Treud). Moreno et al. (1992) found that sage and lavender had high gall ratings and egg mass indices resulting from inoculation with *M. incognita* race 1 and 3 and *M. arenaria*.

The objectives of this research were to: 1) isolate and identify the pathogens causing the die-back disease of sage and lavender, and 2) evaluate the pathogenicity of the suspected pathogens.

## Materials and Methods

### Isolation of *Fusarium oxysporum*

On 20 December, 1991 soil (Loamy, Siliceous, Hyperthermic, Grossarenic Paleudult) was collected from the Green Acres Agronomy farm in an area known to be infested with suspected soilborne pathogens. A die-back disease had severely affected sage and lavender in a fertilization experiment conducted the previous year in the same area (Angell, 1991). The untreated field soil was maintained in dark storage at 10ºC. A portion of the soil was removed from storage and microwaved using the technique described by Ferriss (1984) (Appendix B-1). Fifteen-centimeter-diameter clay pots were disinfected with 20% chlorine solution for 12 hours. Ten clay pots were filled with microwaved soil and five pots were filled with untreated field soil for each herb.

The genotypes of sage and lavender used in this experiment had essential oil compositions of commercial importance. A sage plant, which survived in Angell's (1991) fertilization study, was propagated for use in the study. This sage genotype falls into the chemotype number 2 (Tucker and Maciarello, 1990a; Tucker et al., 1993) (Appendix A-1 and A-2). Cuttings were rooted in a hotbed containing AERO-SOIL® horticultural perlite medium (Chemrock Corp., Grefco Inc., 3435 West Lomita Boulevard, Torrance, CA 90509).

In January 1992, healthy appearing 1-month-old rooted cuttings of sage and lavender were transplanted into clay pots containing microwaved soil (control) and untreated field soil (Figure 3.1). After transplanting, the pots were placed in a 25% shade, fiberglass greenhouse with evaporative cooling. There was no precise control on temperature or photoperiod. Irrigation was provided as needed; pots were drenched with Peter's 20-20-20 liquid fertilizer twice a month, using 1.5 g liter$^{-1}$ of tap water.

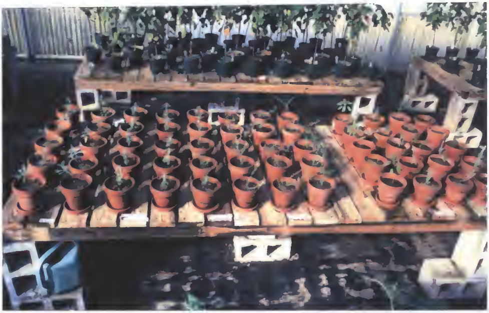

Figure 3.1. Potted herbs growing in untreated and microwaved field soil.

Two potted plants of sage and two of lavender with symptoms of reduced size or yellowing were sacrificed after approximately 8 months of growth. The roots were plated on potato dextrose agar containing 1 ml of tergitol, 100 mg of streptomycin and 50 mg of chlortetracycline per liter of medium (Steiner and Watson, 1965). Eighteen different colonies of *Fusarium* spp. were isolated and later identified as *F. oxysporum* based on characteristics described by Nelson et al. (1983). Two aggressive isolates, isolate 5 from sage and isolate 2 from lavender were grown on Komada's (1975) specific medium for *F. oxysporum* to establish axenic cultures (Appendix B-2).

*Fusarium oxysporum* population density in untreated soil was verified by plating soil on Komada's (1975) medium. Untreated soil collected from pots with severely affected plants yielded 2,400 propagules per gram (ppg) of soil of *F. oxysporum*. Soil from pots containing plants with mild symptoms had 820 ppg of soil. Colonies of *F. oxysporum* were not detected in microwaved soil.

After growth on Komada's (1975) medium, the isolates obtained from the roots were mass produced in wheat seeds following the procedure described by Nelson et al. (1986) (Appendix B-3). Fifteen kilograms of soil from the Green Acres Agronomy Farm were microwaved, infested with isolates 2 or 5 at a ratio of 10 g of inoculated wheat seeds to 1 kg of soil, and stored in polyethylene bags. The infested soil was kept in the dark at 20ºC for 1 month. Bags were kneaded frequently to ensure uniform colonization of the soil and to avoid clumps with high concentration of the fungus.

The soil was plated on Komada's (1975) medium following 1 month of incubation to determine the population. The resulting populations were 15,900 ppg for isolate 5 and 6,900 ppg for isolate 2. These established populations were designated the high concentration for each isolate and were diluted with microwave-treated soil to establish soils with lower populations for use in the preliminary and subsequent pathogenicity tests.

## Preliminary Pathogenicity Test

On 12 December 1992, two tests were conducted to evaluate the pathogenicity of isolate 5 of *F. oxysporum* on sage and isolate 2 on lavender. The tests were established in an air-conditioned glasshouse. A randomized complete block design was used with 10 replications for each test. Inoculum levels of isolate 5 for sage were the following: 1) full strength (15,900 ppg), 2) high density (3,000 ppg), 3) low density (300 ppg), and 4) control (microwaved soil with 0 ppg). Inoculum levels of isolate 2 for lavender were the same with exception that full strength level was 6,900 ppg. Infested soil for sage and lavender was placed in autoclaved 15-cm-diameter clay pots.

Healthy cuttings of sage and lavender were transplanted into pots. Plant height was recorded at the time of transplanting, 30 days after establishment and at harvest (60 days after establishment). Shoot and root fresh weight data were collected at harvest, and plant roots were evaluated for *F. oxysporum* infection. The rating system used is as follows: 1 = white healthy looking roots without adventitious roots; 2 = white healthy looking roots plus adventitious roots; 3 = white healthy looking roots plus adventitious roots and yellowish plant foliage; 4 = some brown roots plus adventitious roots and yellowish and dwarf plants; 5 = many brown roots without adventitious roots, and dwarfed and wilted plants; and 6 = mostly brown roots without adventitious roots, and dying plants (Appendix C-2).

Statistical analysis included ANOVA for all variables measured for each herb. Single-degree of freedom orthogonal (SDFO) contrasts (type 1) were conducted between the control and the three inoculum levels for both tests, and a SDFO contrast (type 2) between the control and the full strength inoculum level was evaluated for the lavender test.

### Differential Host Test

In May 1994, a differential host test, as described by Taylor and Sasser (1980), was conducted in a glasshouse to identify the species and race of root-knot nematode (*Meloidogyne* spp) found to produce galls in field grown sage. No galls were found on field grown lavender. Galled roots of sage were incubated on a Baermann Funnel (Rodríguez-Kábana and Pope, 1981) (Appendix B-4) for 7 days to collect second-stage juveniles (J2).

Seedlings of cotton (*Gossypium hirsutum*) 'Deltapine 51,' pepper (*Capsicum frutescens*) 'California Wonder,' tobacco (*Nicotiana tabacum*) 'NC 95' and tomato (*Lycopersicon esculentum* Mill) 'Rutgers' were transplanted into autoclaved pure sand. Five pots of each host plant were inoculated with 1,000 J2 of root-knot nematode by injecting 10 ml of nematode suspension into each pot. Root gall and egg mass indices were recorded at 60 days using a 0-5 scale (Taylor and Sasser, 1978), where 0 = 0 galls, 1 = 1-2 galls, 2 = 3-10 galls, 3 = 11-30 galls, 4 = 31-100 galls, and 5 = > 100 galls per root system per plant.

## Results and Discussion

### Preliminary Pathogenicity Test with Sage

Highly significant differences were obtained between the control and the three levels of *F. oxysporum* ($P \leq 0.01$) for almost all variables being evaluated, except for percent dead plants (Table 3.1) Plants grown in soil with 300 ppg of *F. oxysporum* had a mean height of 5.6 cm, or 51% of the control (11.0 cm), at 30 days after establishment, and a height of 6.1 cm, or 35.3% of the control (17.3 cm), at 60 days after establishment (harvest). Plants grown in soil with 300 ppg of the pathogen had an average of 4.9 root rating, compared to a rating of 2.6 for control plants. Average root fresh weight and shoot fresh weight of control plants was 3.3% greater than those harvested from the 300 ppg soil treatment.

A single-degree of freedom orthogonal contrast between the control and the three inoculum levels (type 1) was highly significant for plant height at 30 and 60 days after establishment, *F. oxysporum* root rating, shoot fresh weight and root fresh weight ($P \leq 0.01$) (Table 3.1). Also, contrast type 1 was significant for percent dead plants ($P \leq 0.05$). These results provided strong evidence that *F. oxysporum* is

Table 3.1. Analysis of variance summary and single degree of freedom contrasts for the preliminary pathogenicity test with sage.

| *Fusarium oxysporum* (ppg)[†] | Variables | | | | | |
|---|---|---|---|---|---|---|
| | Height | | Dead plants | Root rating | Shoot fresh wt. | Root fresh wt. |
| | 30 days | 60 days | | | | |
| | ----- cm ----- | | - % - | | ------- g ------- | |
| 15,900 | 5.1 | 4.8 | 70 | 5.6 | 0.4 | 1.2 |
| 3,000 | 5.1 | 8.1 | 50 | 4.9 | 0.6 | 2.1 |
| 300 | 5.6 | 6.1 | 50 | 4.9 | 0.7 | 1.7 |
| 0 | 11.0 | 17.3 | 20 | 2.6 | 2.3 | 5.5 |
| ANOVA[‡] | * *[♯] | * * | n s | * * | * * | * * |
| CV (%) | 47 | 96 | 34 | 89 | 100 | 103 |
| Contrast 1[§] | * * | * * | * | * * | * * | * * |

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[†] ppg= propagules per gram of soil, 0 ppg = control.

[‡] ANOVA of a randomized complete block design with 10 replications.

[§] Single degree of freedom contrast between all inoculum levels and the control.

[♯] n s = non significant; ** = significant at $P \leq 0.01$, and * = significant at $P \leq 0.05$.

pathogenic to sage, even at low inoculum levels (Figure 3.2).

Preliminary Pathogenicity test with Lavender

Highly significant differences were obtained between the control and the three inoculum levels of *F. oxysporum* for root rating in lavender ($P \leq 0.01$) (Table 3.2). There were no differences in all other variables.

Table 3.2. Analysis of variance summary and single degree of freedom contrasts for the preliminary pathogenicity test with lavender.

| *Fusarium oxysporum* (ppg)[†] | Variables | | | | | |
|---|---|---|---|---|---|---|
| | Height | | Dead | Root | Shoot | Root |
| | 30 days | 60 days | plants | rating | fresh wt. | fresh wt. |
| | ----- cm ----- | | - % - | | ------- g ------- | |
| 6,900 | 9.2 | 14.5 | 40 | 4.5 | 1.2 | 4.9 |
| 3,000 | 9.6 | 16.4 | 20 | 2.9 | 1.9 | 6.8 |
| 300 | 10.8 | 19.4 | 0 | 2.5 | 1.5 | 6.5 |
| 0 | 11.4 | 19.2 | 0 | 1.8 | 1.6 | 6.4 |
| ANOVA[‡] | n s[#] | n s | n s | * * | n s | n s |
| CV | 26 | 31 | 237 | 56 | 78 | 68 |
| Contrast 1[§] | n s | n s | n s | * | * | * |
| Contrast 2[¶] | n s | * | n s | * | n s | n s |

† ppg= propagules per gram of soil, 0 ppg = control.

‡ ANOVA of a randomized complete block design with 10 replications .

§ Single degree of freedom contrast between all inoculum levels and the control.

¶ Single degree of freedom contrast between the control and full strength (6,900 ppg).

# n s = non significant; ** = significant at $P \leq 0.01$, and * = significant at $P \leq 0.05$.

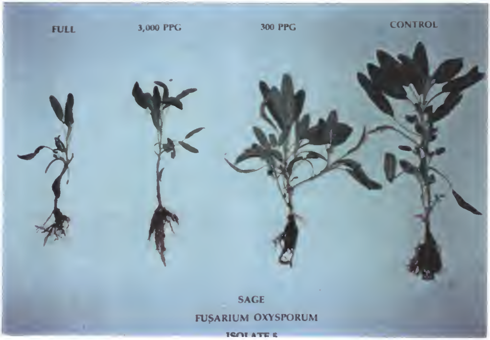

Figure 3.2. Effect of inoculum levels of *Fusarium oxysporum* on plant development in the preliminary pathogenicity test with sage. Treatments are: full strength (15,900 propagules per gram), 3,000 ppg, 300 ppg and control (0 ppg).

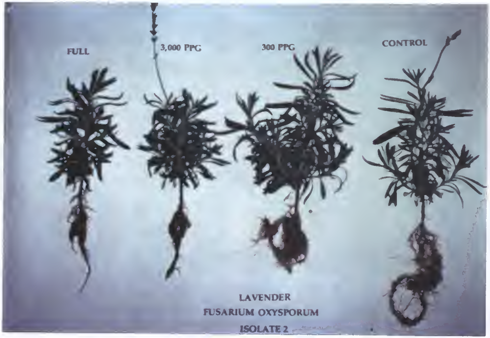

Figure 3.3. Effect of inoculum levels of *Fusarium oxysporum* on plant development in the preliminary pathogenicity test with lavender. Treatments are: full strength (6,900 propagules per gram), 3,000 ppg, 300 ppg and control (0 ppg).

Contrasts between the control and all inoculum levels combined (contrast type 1) were significant for root rating, root fresh weight, and shoot fresh weight ($P \leq 0.05$). Contrasts between the control and the high inoculum level (contrast type 2) were significant for plant height at 60 days after establishment, and root rating ($P < 0.05$). There were no differences between the other variables. No plants were killed at 300 ppg. These results indicate that lavender is less affected by *F. oxysporum* than sage at low inoculum levels (Figure 3.3). Inoculum levels of 6,900 ppg reduced lavender plant height.

## Differential Host Test

Pepper 'California Wonder,' as the differential host plant, had a gall index of 4.2 and an egg mass index of 3.0 (Table 3.3). Tomato 'Rutgers,' the indicator plant, had gall and egg-mass indices of 5.0. Cotton, peanut and tobacco did not exhibit galls or egg masses in their roots. The findings indicate that *M. incognita* race 1 is the root-knot nematode producing galls in field grown sage. These results agree with Moreno et al. (1992), who documented the pathogenicity of *M. incognita* race 1 on sage in greenhouse studies.

Table 3.3. Response of differential host plants to the root-knot nematode isolated from diseased sage plants.

| Differential Host plant cultivar | Gall rating[†] | Egg mass rating[‡] |
|---|---|---|
| Cotton 'Deltapine 51' | 0 | 0 |
| Peanut 'Florunner' | 0 | 0 |
| Pepper 'California wonder' | 4.2 | 3.0 |
| Tobacco 'NC 95' | 0 | 0 |
| Tomato 'Rutgers' | 5.0 | 5.0 |

[†] Gall rating: 0 = 0 galls, 1 = 1-2 galls, 2 = 3-10 galls, 3 = 11-30 galls, 4 = 31-100 galls, and 5 = > 100 galls per root system per plant.

[‡] Egg mass rating: 0 = no egg mass present, 1 = 1 to 2, 2 = 3 to 10, 3 = 11 to 30, 4 = 31 to 100, and 5 = more than 100 egg masses per plant.

# CHAPTER IV

# COMBINED PATHOGENICITY TESTS WITH *Fusarium oxysporum* and *Meloidogyne incognita* ON SAGE

## Introduction

Tucker and Maciarello (1990b) considered that, under hot and humid conditions, sage (*Salvia officinalis* L.) becomes susceptible to soilborne fungi. *Fusarium* spp. were isolated from sage plants grown at the University of Florida from 1989 through 1990. Sage plants appeared stunted with root decay and black lesions extending up the main stem from the soil line. Espaillat et al. (1993) obtained 18 isolates of *Fusarium oxysporum* Link from plants grown in a glasshouse in infested soil. Searches of the published literature conducted by the senior author and by Simon et al. (1984) failed to find previous reports of disease caused by *F. oxysporum* on sage.

Hasseb and Pandley (1987) concluded that root-knot nematodes (*Meloidogyne* spp.) are an important constraint in the production of several medicinal and aromatic plants. Lisetskaya (1973) diagnosed *M. hapla* Chitwood in roots of sage. Ibrahim et al. (1983), in a nematode screening test, found sage to be susceptible to *M. arenaria* (Neal) Chitwood race 1, *M. incognita* (Kofoid and White) Chitwood races 1 and

2, and *M. javanica* (Treub) Chitwood. Moreno et al. (1992) found sage plants to have high gall ratings and egg mass indices resulting from inoculation with *M. incognita* race 1.

Webster (1985) documented that *F. oxysporum*, alone and in interaction with *M. incognita*, is capable of reducing plant populations and yield of many crops. Results of studies on interactions of *F. oxysporum* and *M. incognita* in tomato (*Lycopersicon esculentum* L.) and cotton (*Gossypium hirsutum* L.) are contradictory and have not been verified (Abawai and Barker, 1984, and Sikora and Carter, 1987). Martyn et al. (1986) obtained inconclusive results on the synergistic or additive interaction of *F. oxysporum* and root-knot nematodes in cotton. No studies have been published on diseases caused by interaction of soilborne pathogens in sage.

Powell (1971) noted that root-knot nematode damage declines due to the lack of suitable root tissue following infection by another soilborne pathogen. Bruehl (1987b) supported Powell's claim, suggesting that the organism accompanying the nematode may gain from an interaction at the expense of the nematode. Few pathogenicity studies have been designed with *F. oxysporum* as the primary pathogen and *M. incognita* as the secondary pathogen (Dropkin, 1980, and Mountain and McKeen, 1962). The objecives of this study were to determine the roles of *F. oxysporum*, *M. incognita* race-1, and the interaction of the two organisms on growth and dieback of sage.

## Materials and Methods

### Preparation of Inoculum

Sage roots taken from field grown plants exhibiting symptoms of reduced size and yellowing were plated on potato dextrose agar supplemented with 1.0 ml of tergitol, 100 mg of streptomycin and 50 mg of chlortetracycline per liter of medium (Steiner and Watson, 1965). An aggressive isolate of *F. oxysporum* was identified (Nelson et al., 1983), grown on Komada's (1975) specific medium for *F. oxysporum*, and mass produced in wheat seeds (Nelson et al., 1986). Soil classified as Arredondo fine sand (loamy siliceous, hyperthermic family of Arenic and Grossarenic, Paleudults) was microwaved (Ferriss, 1984), infested with the isolate at a ratio of 10 g of inoculated wheat seeds to 1 kg of soil, and then stored in polyethylene bags in the dark at 20ºC for a month. Bags were kneaded frequently to ensure uniform colonization of the soil and to avoid clumps with high concentrations of fungus. Following storage, samples of soil from the bags were plated on Komada's (1975) medium to establish the population density, which had attained 15,900 propagules per gram (ppg) of soil of *F. oxysporum*. This population was designated the high concentration and was diluted with microwave-treated soil to create treatment levels used in the combined pathogenicity tests.

Second stage juveniles (J2) of *M. incognita* race 1 were isolated from galled roots of field grown sage plants and identified in a differential host test (Jenkins, 1964) (Chapter III). The J2 were produced in pepper (*Capsicum frutescens* L.) 'California Wonder' for 3 months, and hatched using the Baermann funnel incubation method (Rodríguez-Kábana, 1981).

Two combined pathogenicity tests were initiated in summer of 1994 using $3^2$ factorial experiments in a randomized complete block design with 10 replications. The treatment factors included three inoculum levels of *M. incognita* race 1 (0, 100 and 1,000 J2 per ml) and three inoculum levels of *F. oxysporum* (0, 200 and 2,000 ppg of soil). The first pathogenicity test (test 1) was established on 29 July 1994 in an evaporative-cooled glasshouse, and the second pathogenicity test (test 2) began on 13 August 1994 in a refrigerated-air-cooled glasshouse.

Healthy appearing, 1-month-old rooted cuttings of sage were transplanted into autoclaved, 10-cm clay pots containing soil with 0, 200, or 2,000 ppg of *F. oxysporum*. Each pot was located on a water retention plate with a 10-cm spacing between pots to avoid water splash and contamination among treatments (Figure 4.1).

Ten days after transplant, 10 ml of a suspension of 0, 200, or 2,000 J2 of M. incognita race 1 $ml^{-1}$ were injected into the appropriate treatment pot. Pots were watered as needed and drenched with Peter's® 20-20-20 liquid fertilizer every 2 weeks using 1.5 g $liter^{-1}$ of tap water.

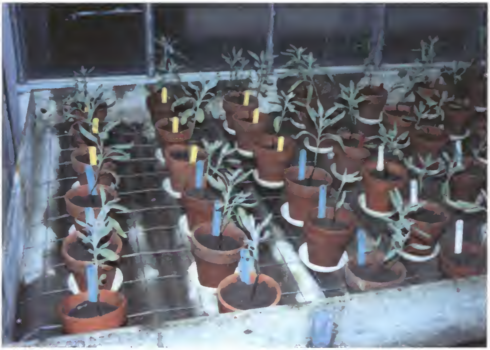

Figure 4.1. First combined pathogenicity test with sage 7 days after establishment.

Minimum and maximum temperatures were recorded daily and the bi-weekly mean temperature was calculated (Appendix D-3). Three spray applications of Diazinon® were required in test 2 for control of greenhouse white flies (*Trialeurodes vaporariorum* (Westwood) and cottony cushion mealy bugs (*Icerya purchasi* Maskell). Plants in test 1 and 2 were harvested at 291 and 300 days, respectively, after establishment.

Data collection

The percentages of dead plants were assessed for 10 replications, eight times during the course of test 1 and test 2 (Appendix D-1). A total of six plant height measurements over time were recorded during the course of test 1 and test 2 (Appendix D-2). Plant height was measured from the soil-line at the base of the stem to the tip of the highest leaf.

Three replications across treatments were harvested in test 1 on 1 December (123 days after establishment). Plant response data obtained from this destructive sampling and also from five replications harvested at final harvest (291 days after establishment) included the following parameters: internode length, canopy width, stem diameter, shoot dry weight, root dry weight, *F. oxysporum* root rating, number of propagules of *F. oxysporum* per gram (ppg) of soil, percent *F. oxysporum* infection of main and fibrous roots, *M. incognita* gall rating, and number of *M. incognita* J2 100 $ml^{-1}$ of soil.

In test 2, internode length, number of axillary branches, and number of flowering plants were collected for all treatments at 123 days after establishment. Number of flowering plants was again recorded at 232 days after establishment. Final harvest of five replications took place at 300 days after establishment. The parameters measured were the same as those described for the destructive sampling in test 1 and included a final count of dead plants and plant height in both tests.

Internode length was calculated by dividing the length of the main stem by the number of branches. Canopy width was measured using circular templates of varying diameters; the template diameter which best fit around the plant canopy at the widest point was recorded (Appendix C-1). Stem diameter was measured at the soil-line with a caliper. Following harvest, shoots (all above ground vegetation) and roots were separated and dried at 70ºC for 4 days in a forced-air oven.

Pathogen and disease parameters recorded at harvest included *F. oxysporum* root rating, number of propagules of *F. oxysporum* per gram (ppg) of soil, percent *F. oxysporum* infection of main and fibrous roots, *M. incognita* gall rating, and number of *M. incognita* J2 100 $Ml^{-1}$ soil.

Root necrosis and decay, resulting from *F. oxysporum*, were rated as follows on a scale of 1-6: 1 = white, healthy looking roots without adventitious roots; 2 = white, healthy looking roots with adventitious roots; 3 = white, healthy looking roots with adventitious roots and yellowish plant foliage; 4 = some brown roots with adventitious roots and yellowish and dwarf plants; 5 = many brown roots without adventitious roots, and dwarf and wilted plants; and 6 = mostly brown roots without adventitious roots, and dying plants (Appendix C-2). In the second test, a destructive sample was not taken.

The percent *F. oxysporum* infection in the main and fibrous roots was determined by plating a subsample of seven, 1.3-cm-long root pieces on Komada's (1975) medium, incubating the roots at 27ºC with light and dark periods cycled every 12 hrs, and counting the number of infected root pieces after 7 days.

*Meloidogyne incognita* gall rating and number of *M. incognita* J2 100 $ml^{-1}$ of soil were recorded for all harvested plants. Gall ratings were recorded as follows: 0 = 0 galls, 1 = 1-2 galls, 2 = 3-10 galls, 3 = 11-30 galls, 4 = 31-100 galls, and 5 = more than 100 galls per root system per plant (Taylor and Sasser, 1978). The *M. incognita* J2 in a sample of 100 ml soil were separated from soil using the centrifugal flotation procedure (Jenkins, 1964), and counted using a compound microscope.

## Statistical Analysis

Statistical analysis included analysis of variance (ANOVA) for a $3^2$ factorial experiment arranged in a randomized complete block design. Simple linear correlations between all variables were calculated for all harvest data. Logarithmic transformation [Log(x+1)] was done on the following data: number of *F. oxysporum* ppg of soil and number of *M. incognita* J2 100 $ml^{-1}$ soil obtained from the destructive sampling in test 1, and harvest data from both tests. The Michigan State University Statistical Program (MSTAT-C, 1992) was used to analyze all data.

## Results and Discussion

### Measurements over Time

The data collected over time in both pathogenicity tests showed no consistent effect of *F. oxysporum* and *M. incognita* on the percent dead plants and no evidence of interaction between pathogens in test 1 and test 2 (Tables 4.1 and 4.2). Less than 7% mortality was detected in either test over the 300-day study period.

Plants grown in soil infested with *F. oxysporum* demonstrated an etiolation effect early in both tests (Tables 4.3 and 4.4). Plant height increased with increasing inoculum levels of *F. oxysporum* at 123 days in both tests. In test 1, plant height means of 36.5, 42.0 and 42.6 cm ($P \leq 0.10$) were calculated from *F. oxysporum* levels (0, 200, and 2,000 ppg of soil) averaged across nematode levels at 123 days (Table 4.3). Corresponding plant height means at 123 days in test 2 of 38.1, 49.9, and 52.1 cm were observed ($P \leq 0.01$) (Table 4.4). The reduction of mean plant height with *M. incognita* inoculum levels of 0, 100, and 1,000 J2 $ml^{-1}$ of soil, averaged across *F. oxysporum* levels (0, 200, and 2,000 ppg), was consistent between test 1, with 51.4, 38.2, and 46.5 cm ($P \leq 0.10$), respectively, and test 2,

Table 4.1 Means and ANOVA summary of the effects of *Fusarium oxysporum* and *Meloidogyne incognita* inoculum levels on the percent dead plants in the first pathogenicity test with sage[†].

| Pathogens | | Days after establishment | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Fusarium oxysporum* | *Meloidogyne incognita* | 14 | 38 | 72 | 99 | 122 | 178 | 232 | 291 |
| ppg[§] | J2 | percent dead plants[‡] | | | | | | | |
| 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 3 |
| 0 | 100 | 0 | 3 | 3 | 4 | 6 | 6 | 6 | 6 |
| 0 | 1000 | 0 | 0 | 1 | 4 | 4 | 4 | 6 | 6 |
| 200 | 0 | 0 | 0 | 1 | 3 | 3 | 3 | 3 | 3 |
| 200 | 100 | 0 | 1 | 3 | 3 | 3 | 4 | 6 | 6 |
| 200 | 1000 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 2000 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| 2000 | 100 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 |
| 2000 | 1000 | 0 | 0 | 3 | 3 | 3 | 3 | 3 | 3 |
| ANOVA: | | | | | | | | | |
| Fusarium (A) | | -[¶] | n s[#] | - | - | n s | - | n s | n s |
| Meloidogyne (B) | | - | n s | - | - | n s | - | n s | * |
| Interaction (AXB) | | - | n s | - | - | n s | - | n s | n s |
| CV (%) | | - | 291 | - | - | 179 | - | 156 | 187 |

[†] Data at all dates analyzed with ANOVA as a $3^2$ Factorial.
[‡] Percent dead plants at specific dates after establishment of the experiment.
[§] ppg = propagules per gram of soil, and J = juveniles 100 $ml^{-1}$ of soil.
[¶] No ANOVA was done at dates with - sign.
[#] n s = F value non significant, * = F value significant at $P \leq 0.05$.

Table 4.2. Means and ANOVA summary of the effects of *Fusarium oxysporum* and *Meloidogyne incognita* inoculum levels on the cumulative percent of dead plants in the second pathogenicity test with sage[†].

| Pathogens | | Days after establishment | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Fusarium oxysporum* | *Meloidogyne incognita* | 14 | 38 | 72 | 99 | 123 | 178 | 232 | 300 |
| ppg[§] | J2 | percent dead plants[‡] | | | | | | | |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| 0 | 100 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 |
| 0 | 1000 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 200 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 200 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 200 | 1000 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 2000 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2000 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 2000 | 1000 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 2 |
| ANOVA: | | | | | | | | | |
| Fusarium (A) | | -[¶] | - | - | - | - | - | - | n s[#] |
| Meloidogyne (B) | | - | - | - | - | - | - | - | n s |
| Interaction (AXB) | | - | - | - | - | - | - | - | n s |
| CV (%) | | - | - | - | - | - | - | - | 187 |

[†] data at all dates analyzed with ANOVA as a $3^2$ Factorial.

[‡] Cumulative percent dead plants at specific dates after establishment of the experiment.

[§] ppg = propagules per gram of soil, and J = juveniles 100 $ml^{-1}$ of soil.

[¶] No ANOVA was done at dates with the - sign.

[#] n s = F value non significant.

Table 4.3. Means and ANOVA summary of the effects of *Fusarium oxysporum* and *Meloidogyne incognita* inoculum levels on plant height in the first pathogenicity test with sage.[†]

| Pathogens | | Days after establishment | | | | | |
|---|---|---|---|---|---|---|---|
| *Fusarium oxysporum* | *Meloidogyne incognita* | 2 | 44 | 123 | 204 | 247 | 287 |
| ppg[§] | J2 | plant height (cm)[‡] | | | | | |
| 0 | 0 | 19.1 | 30.4 | 37.1 | 43.0 | 44.2 | 40.0 |
| 0 | 100 | 20.3 | 29.8 | 35.4 | 38.3 | 33.7 | 26.0 |
| 0 | 1000 | 19.8 | 29.9 | 36.9 | 44.8 | 45.9 | 49.3 |
| 200 | 0 | 19.1 | 35.3 | 45.3 | 56.4 | 59.1 | 64.7 |
| 200 | 100 | 18.9 | 36.8 | 41.7 | 46.0 | 35.3 | 26.7 |
| 200 | 1000 | 19.2 | 34.6 | 39.0 | 42.3 | 42.8 | 44.7 |
| 2000 | 0 | 20.4 | 36.9 | 44.6 | 51.3 | 50.8 | 48.3 |
| 2000 | 100 | 20.9 | 34.3 | 40.2 | 44.3 | 45.5 | 47.2 |
| 2000 | 1000 | 20.2 | 34.0 | 43.1 | 50.8 | 50.9 | 51.7 |
| ANOVA: | | | | | | | |
| Fusarium (A) | | n s[#] | * * | ¥ | –[¶] | n s | n s |
| Meloidogyne (B) | | n s | n s | n s | – | ¥ | * |
| Interaction (AXB) | | n s | n s | n s | – | n s | n s |
| CV (%) | | 20 | 55 | 79 | – | 85 | 33 |

[†] Data at all dates analyzed with ANOVA as a $3^2$ Factorial.

[‡] Percent plant height measured from the soil-line to the tip of the highest leaf taken at specific dates after establishment of the experiment.

[§] ppg = propagules per gram of soil, and J = juveniles 100 $ml^{-1}$ of soil.

[¶] No ANOVA was done at dates with the - sign.

[#] n s = F value non significant; ** = F value significant at $P \le 0.01$; * = F value significant at $P \le 0.05$, ¥ = F value significant at $P \le 0.10$.

Table 4.4. Means and ANOVA summary of the effects of *Fusarium oxysporum* and *Meloidogyne incognita* inoculum levels on plant height in the second pathogenicity test with sage.[†]

| Pathogens | | Days after establishment | | | | | |
|---|---|---|---|---|---|---|---|
| *Fusarium oxysporum* | *Meloidogyne incognita* | 2 | 44 | 123 | 204 | 247 | 287 |
| ppg[§] | J2 | plant height (cm)[‡] | | | | | |
| 0 | 0 | 20.2 | 28.8 | 36.8 | 41.6 | 45.3 | 54.4 |
| 0 | 100 | 19.1 | 27.4 | 37.2 | 42.5 | 46.9 | 53.7 |
| 0 | 1000 | 19.1 | 29.6 | 40.2 | 45.6 | 49.3 | 56.8 |
| 200 | 0 | 19.6 | 50.4 | 50.9 | 54.2 | 61.8 | 69.2 |
| 200 | 100 | 18.9 | 45.7 | 48.7 | 52.1 | 48.0 | 45.6 |
| 200 | 1000 | 18.1 | 48.0 | 50.1 | 58.4 | 71.2 | 80.4 |
| 2000 | 0 | 21.1 | 47.6 | 52.6 | 59.1 | 66.0 | 78.6 |
| 2000 | 100 | 21.9 | 47.5 | 51.6 | 54.5 | 63.8 | 65.2 |
| 2000 | 1000 | 20.2 | 47.0 | 52.0 | 54.1 | 47.9 | 42.8 |
| ANOVA: | | | | | | | |
| Fusarium (A) | | n s[¶] | - | * * | -[¶] | n s | n s |
| Meloidogyne (B) | | n s | - | n s | - | * | n s |
| Interaction (AXB) | | n s | - | n s | - | n s | * |
| CV (%) | | 15 | - | 40 | - | 85 | 35 |

[†] Data at all dates analyzed with ANOVA as a $3^2$ Factorial.

[‡] Cumulative plant height measured from the soil line to the tip of the highest leaf taken at specific dates after establishment of the experiment.

[§] ppg = propagules per gram of soil, and J = juveniles 100 $ml^{-1}$ of soil.

[¶] No ANOVA was done at dates with the - sign.

[¶] n s = F value non significant; ** = F value significant at $P \le 0.01$; and * = F value significant at $P \le 0.05$.

with 57.7, 52.9, and 56.1 cm ($P \leq 0.05$), respectively, at 247 days after establishment (Tables 4.3 and 4.4). Thus, the early stimulatory effect of *F. oxysporum* on plant height observed at 123 days gave way to a trend of decreased plant height in response to *M. incognita* at the end of the tests, especially at the 100 J2 inoculum level.

Plant height data collected immediately prior to harvest (287 days) were not consistent in test 1 and 2. In test 2, plant height data indicated an interaction effect between pathogens ($P \leq 0.05$) (Table 4.4). Plant height increase appeared to be a function of *F. oxysporum* inoculum levels at *M. incognita* inoculum levels of 0 and 1,000 J2 100 $ml^{-1}$ of soil.

### First Destructive Sample

Data obtained from the initial destructive sample (123 days after establishment) of three replications in test 1 showed a pattern of increasing pathogen population densities consistent in most cases with the imposed treatments (Tables 4.5 and 4.6). Despite the presence of pathogens at 123 days, there was no significant effect on measured plant parameters, except for internode length and canopy width. Internode length increased as *F. oxysporum* inoculum levels increased in both tests (Tables 4.5 and 4.7). These results support the etiolation effect observed in the plant height data at 123 days after establishment.

Table 4.5. Treatment means and analysis of variance summary for agronomic and *Meloidogyne incognita* data collected in the first destructive sample of the first pathogenicity test with sage[†].

| Pathogens | | Agronomic variables[‡] | | | | | *M. incognita* variables[§] | | |
|---|---|---|---|---|---|---|---|---|---|
| *Fusarium oxysporum* | *Meloidogyne incognita* | Internode length | Canopy Width | Stem Diam. | Dry Weight | | Gall Rating | J2 in Soil | Log J2 in Soil |
| | | | | | Shoot | Root | | | |
| ppg[¶] | J2 | ------ cm | ------ | -mm- | ----- g | ----- | | -no.- | |
| 0 | 0 | 1.4 | 18.2 | 4.2 | 14.7 | 18.3 | 0 | 0 | 2.2 |
| 0 | 100 | 0.9 | 13.1 | 4.7 | 16.7 | 17.0 | 1.7 | 11 | 3.4 |
| 0 | 1000 | 1.0 | 10.1 | 3.5 | 14.3 | 17.0 | 2.0 | 22 | 3.1 |
| 200 | 0 | 1.2 | 11.9 | 4.0 | 15.0 | 15.7 | 0 | 0 | 3.2 |
| 200 | 100 | 1.4 | 11.6 | 4.3 | 16.0 | 17.7 | 1.7 | 14 | 2.4 |
| 200 | 1000 | 1.4 | 14.6 | 4.6 | 16.0 | 19.7 | 2.7 | 78 | 2.3 |
| 2000 | 0 | 1.6 | 12.7 | 4.2 | 15.7 | 17.7 | 0 | 0 | 2.2 |
| 2000 | 100 | 1.5 | 14.0 | 4.8 | 15.3 | 18.0 | 3.3 | 82 | 1.3 |
| 2000 | 1000 | 1.4 | 13.8 | 4.7 | 16.0 | 18.3 | 2.7 | 42 | 2.9 |
| ANOVA: | | | | | | | | | |
| Fusarium (A) | | ¥[♯] | n s | n s | n s | n s | n s | n s | ¥ |
| Meloidogyne (B) | | n s | n s | n s | n s | n s | * * | * | n s |
| Interaction (AXB) | | n s | ¥ | n s | n s | n s | n s | n s | * |
| CV (%) | | 51 | 49 | 21 | 8 | 14 | 85 | 127 | 29 |

† All variables were analyzed for ANOVA as a $3^2$ Factorial.

‡ Agronomic variables: internode length obtained by dividing total stem length by the number of branches; canopy width determined using a template; stem diameter measured at the soil-line; dry weights = dried for 3 days at 70ºC.

§ *Meloidogyne incognita* variables: Gall rating based on a scale of 0-5; J2 in soil = number of J2 of *M. incognita* race 1 in 100 $ml^{-1}$ of soil; log J2 in soil = logarithm of x+1 of the number of J2 in soil.

¶ ppg = number of propagules per gram of soil, and J2 = juveniles 100 $ml^{-1}$ of soil.

♯ n s = F value non significant; ** = F value significant at $P \leq 0.01$; * = F value significant at $P \leq 0.05$, ¥ = F value significant at $P \leq 0.10$.

Table 4.6. Treatment means and analysis of variance summary for *Fusarium oxysporum* data collected in the first destructive sample of the first pathogenicity test with sage[†].

| Pathogens | | *Fusarium oxysporum* variables[‡] | | | | |
|---|---|---|---|---|---|---|
| *Fusarium oxysporum* | *Meloidogyne incognita* | Root Rating | ppg in soil | | Root infection | |
| | | | Amount | Log | Main | Fibrous |
| ppg[§] | J2 | | | | --------- % | --------- |
| 0 | 0 | 2.3 | 97 | 0.8 | 0 | 10.3 |
| 0 | 100 | 1.7 | 47 | 0.7 | 0 | 0 |
| 0 | 1000 | 2.0 | 240 | 1.7 | 0 | 0 |
| 200 | 0 | 2.3 | 383 | 2.5 | 0 | 24.8 |
| 200 | 100 | 2.0 | 380 | 2.5 | 0 | 24.1 |
| 200 | 1000 | 1.0 | 2333 | 1.3 | 33.3 | 24.4 |
| 2000 | 0 | 1.3 | 240 | 2.4 | 33.3 | 29.1 |
| 2000 | 100 | 1.7 | 807 | 2.9 | 16.7 | 47.9 |
| 2000 | 1000 | 1.7 | 523 | 2.7 | 33.3 | 52.9 |
| ANOVA: | | | | | | |
| Fusarium (A) | | n s[¶] | n s | * | n s | * * |
| Meloidogyne (B) | | n s | n s | n s | n s | n s |
| Interaction (AXB) | | n s | n s | n s | n s | n s |
| CV (%) | | 53 | 239 | 56 | 275 | 84 |

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† All variables were analyzed for ANOVA as a $3^2$ Factorial.

‡ *Fusarium oxysporum* variables: root rating based on a scale of 1-6; propagules per gram in the soil at harvest; percent of main and fibrous roots infected.

§ ppg = number of propagules per gram of soil, and J2 = juveniles 100 $ml^{-1}$ of soil.

¶ n s = F value non significant; ** = F value significant at $P \leq 0.01$; * = F value significant at $P \leq 0.05$

Table 4.7. Treatment means and analysis of variance summary for additional data collected at 123 days after establishment of the second pathogenicity test with sage[†].

| Pathogens | | Agronomic variables[‡] | | | |
|---|---|---|---|---|---|
| *Fusarium* *oxysporum* | *Meloidogyne* *incognita* | Internode length | Axillary branches | Flowering plants | |
| | | | | 123 | 232 |
| ppg[§] | J2 | - cm - | -- no.-- | -------- no. | --------- |
| 0 | 0 | 1.9 | 13.6 | 0.3 | 0.2 |
| 0 | 100 | 1.9 | 10.8 | 0.6 | 0.4 |
| 0 | 1000 | 1.9 | 11.8 | 0.4 | 0.1 |
| 200 | 0 | 2.5 | 11.7 | 0.7 | 0.4 |
| 200 | 100 | 2.3 | 13.8 | 0.4 | 0.2 |
| 200 | 1000 | 2.5 | 16.0 | 0.3 | 0.1 |
| 2000 | 0 | 2.5 | 15.4 | 0.4 | 0.2 |
| 2000 | 100 | 2.5 | 10.7 | 0.1 | 0.1 |
| 2000 | 1000 | 2.5 | 10.5 | 0.3 | 0.1 |
| ANOVA: | | | | | |
| Fusarium (A) | | * *[¶] | n s | n s | n s |
| Meloidogyne (B) | | n s | n s | n s | n s |
| Interaction (AXB) | | n s | n s | n s | n s |
| CV (%) | | 29 | 53 | 205 | 123 |

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[†] All variables were analyzed for ANOVA as a $3^2$ Factorial.

[‡] Agronomic variables: internode length obtained by dividing total stem length by the number of branches; axillary branches = counts of the number of axillary branches coming out of the prostrate main stem; flowering plants = the number of plants which had flowers at 123 and 232 days after establishment.

[§] ppg = number of propagules per gram of soil, and J2 = juveniles 100 $ml^{-1}$ of soil.

[¶] n s = F value non significant, ** = F value significant at $P \leq 0.01$

Canopy width in test 1 was not affected by any of the pathogens alone, but did show the effect of interaction between pathogens ($P \leq 0.10$) (Table 4.5). Sage plants growing in soil without infestation with *F. oxysporum* (0 ppg of soil) had a smaller canopy at higher inoculum levels of *M. incognita*. However, with *F. oxysporum* at 200 and 2,000 ppg of soil this trend was not observed.

## Final Harvest

Stem diameter data were not validated by the response in both tests. In test 1, stem diameter was not influenced by the pathogens (Table 4.8), but in test 2 stem diameter decreased with increasing levels of *F. oxysporum* ($P \leq 0.01$) (Table 4.9). In test 2, stem diameter was correlated with *F. oxysporum* inoculum levels ($r = -0.74$, $P \leq 0.01$).

Effects of the nematode on shoot and root dry weights were inconsistent between the two tests (Tables 4.8 and 4.9). In test 1 shoot dry weight was not affected by pathogen levels, but in test 2 shoot dry weight was reduced by increasing inoculum levels of *M. incognita* ($P \leq 0.01$) (Table 4.9). There was an interaction effect resulting in lower shoot dry weight with increasing inoculum levels of both pathogens ($P \leq 0.10$). Shoot dry weight correlated inversely with *F. oxysporum* root rating ($r = -0.72$, $P \leq 0.01$), consistent with an effect of *F. oxysporum* infection on shoot dry weight (Appendix D-5). An inverse correlation of shoot

Table 4.8. Summary of treatment means and analysis of variance for agronomic, *Fusarium oxysporum* and *Meloidogyne incognita* variables in the first pathogenicity test with sage at harvest[†].

| Pathogens | | Agronomic variables[‡] | | | F. oxysporum variables[§] | | | | | *M.incognita* variables[¶] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *F.oxysporum* | *M.incognita* | Stem Diameter | Dry Weight Shoot | Dry Weight Root | Root Rating | ppg in soil Amount | ppg in soil Log | Root infection main | Root infection fibrous | Gall Rating | J2 in Soil | Log J2 Soil |
| ppg[#] | J2 | -mm- | --- g --- | | | - no.- | | ----- % ------ | | | -no.- | |
| 0 | 0 | 6.5 | 24.4 | 21.4 | 1.00 | 333 | 2.46 | 1.7 | 11.7 | 0 | 222 | 2.21 |
| 0 | 100 | 5.9 | 19.9 | 15.8 | 1.33 | 2733 | 2.90 | 46.7 | 10.8 | 3.7 | 2695 | 3.39 |
| 0 | 1000 | 7.2 | 21.4 | 18.3 | 1.33 | 276 | 0.97 | 10.0 | 1.8 | 3.3 | 1491 | 3.10 |
| 200 | 0 | 6.3 | 23.4 | 18.1 | 2.33 | 5600 | 3.32 | 75.0 | 66.6 | 0 | 1937 | 3.19 |
| 200 | 100 | 5.8 | 21.1 | 17.2 | 4.00 | 5800 | 3.73 | 63.3 | 96.5 | 3.3 | 1186 | 2.42 |
| 200 | 1000 | 6.2 | 22.8 | 18.3 | 3.33 | 2467 | 3.31 | 38.3 | 93.3 | 5.0 | 445 | 2.30 |
| 2000 | 0 | 5.3 | 20.6 | 16.8 | 3.67 | 4200 | 3.39 | 95.0 | 92.5 | 0 | 333 | 2.22 |
| 2000 | 100 | 6.5 | 21.9 | 16.7 | 3.33 | 5267 | 3.51 | 90.0 | 99.1 | 3.7 | 120 | 1.27 |
| 2000 | 1000 | 6.0 | 20.8 | 18.5 | 3.00 | 7467 | 3.84 | 96.7 | 99.8 | 5.0 | 1278 | 2.90 |
| ANOVA: | | | | | | | | | | | | |
| Fusarium (A) | | n s[††] | n s | n s | * * | * | * * | * * | * * | n s | n s | ¥ |
| Meloidogyne (B) | | n s | n s | * | n s | n s | n s | n s | n s | * * | n s | n s |
| Interaction (AXB) | | n s | n s | n s | n s | n s | n s | n s | n s | n s | ¥ | * |
| CV (%) | | 19 | 8 | 11 | 43 | 98 | 26 | 42 | 21 | 27 | 109 | 29 |

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[†] All variables analyzed with ANOVA as a $3^2$ Factorial.

[‡] Agronomic variables: stem diameter measured at the soil-line; dry weights after 3 days at 70°C.

[§] *Fusarium oxysporum* variables: root rating based on a scale of 1-6; propagules per gram in the soil at harvest; logarithm (x+1) of number J2 in the soil; and percent of main and fibrous roots infected.

[¶] *Meloidogyne incognita* variables: gall rating based on a scale of 0-5; number of J2 of *M.incognita* race 1 in 100 ml of soil; logarithm J2 in soil of number J2 in soil.

[#] ppg = propagules per gram of soil, and J = juveniles 100 $ml^{-1}$ of soil.

[††] n s = F value non significant; ** = F value significant at $P \le 0.01$; * = F value significant at $P \le 0.05$;

¥ F value significant at $P \le 0.10$.

Table 4.9. Summary of treatment means and analysis of variance for agronomic, *Fusarium oxysporum* and *Meloidogyne incognita* variables in the second pathogenicity test with sage at harvest[†].

| Pathogens | | Agronomic variables[‡] | | | *F. oxysporum* variables[§] | | | | | *M.incognita* variables[¶] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *F.oxy-sporum* | *M.incog-nita* | Stem Diam | Dry Weight | | Root Rating | ppg in soil | | Root infection | | Gall Rating | J2 in Soil | Log J2 |
| | | | Shoot | Root | | Amount | Log | main | fibrous | | | |
| ppg[#] | J2 | -mm- | ---- g | ---- | | -no.- | | ----- % | ------ | | -no.- | |
| 0 | 0 | 6.8 | 24.6 | 20.7 | 1.6 | 1800 | 3.12 | 33.3 | 29.2 | 0 | 486 | 2.42 |
| 0 | 100 | 6.9 | 23.2 | 24.4 | 1.8 | 9067 | 3.76 | 43.3 | 21.7 | 2.3 | 1602 | 2.80 |
| 0 | 1000 | 7.0 | 25.9 | 24.0 | 1.0 | 200 | 0.93 | 8.3 | 3.3 | 3.7 | 403 | 2.32 |
| 200 | 0 | 4.5 | 26.2 | 21.0 | 2.8 | 2600 | 3.40 | 21.7 | 50.0 | 0 | 214 | 1.55 |
| 200 | 100 | 5.1 | 19.3 | 23.2 | 3.2 | 4667 | 3.66 | 51.7 | 74.2 | 3.0 | 757 | 2.68 |
| 200 | 1000 | 5.3 | 24.0 | 21.6 | 3.6 | 12533 | 4.05 | 76.7 | 70.0 | 4.7 | 709 | 2.61 |
| 2000 | 0 | 5.4 | 26.9 | 23.2 | 2.2 | 4667 | 3.59 | 35.0 | 69.2 | 0 | 39 | 1.54 |
| 2000 | 100 | 4.3 | 20.6 | 20.3 | 3.4 | 7200 | 3.82 | 50.0 | 86.7 | 4.7 | 843 | 2.71 |
| 2000 | 1000 | 5.2 | 20.1 | 22.8 | 3.4 | 14133 | 4.03 | 71.7 | 90.8 | 5.0 | 419 | 2.40 |
| ANOVA: | | | | | | | | | | | | |
| Fusarium (A) | | * *[††] | n s | n s | * * | n s | * * | n s | * * | * | n s | n s |
| Meloidogyne (B) | | n s | * * | n s | * | ¥ | n s | n s | n s | * * | ¥ | * |
| Interaction (AXB) | | n s | ¥ | n s | ¥ | n s | * * | n s | * * | n s | n s | n s |
| CV (%) | | 25 | 8 | 20 | 36 | 88 | 19 | 82 | 20 | 33 | 119 | 28 |

[†] All variables analyzed with ANOVA as a $3^2$ Factorial.

[‡] Agronomic variables: stem diameter measured at the soil line; dry weights after 3 days at 70ºC.

[§] *Fusarium oxysporum* variables: root rating based on a scale of 1-6; propagules per gram in the soil at harvest; logarithm (x+1) of number J2 in the soil; and percent of main and fibrous roots infected.

[¶] *Meloidogyne incognita* variables: gall rating based on a scale of 0-5; number of J2 of *M.incognita* race 1 in 100 ml of soil; logarithm J2 in soil of number J2 in soil.

[#] ppg = propagules per gram of soil, and J = juveniles 100 $ml^{-1}$ of soil.

[††] n s = non significant; ** = significant at $P \leq 0.01$; * = significant at $P \leq 0.05$, ¥ = significant at $P \leq 0.10$.

weight with number of J2 100 $ml^{-1}$ of soil ($r = -0.59$, $P \leq 0.01$) is consistent with a nematode effect on shoot weight.

In test 1, root dry weight was reduced at 100 J2 of *M. incognita* $ml^{-1}$ soil across *F. oxysporum* levels ($P \leq 0.05$). However, in test 2, root dry weight was not affected by the pathogens (Table 4.9). Root dry weight correlated with infection of main roots by *F. oxysporum* in both, test 1 ($r = -0.40$, $P \leq 0.05$) and test 2 ($r = 0.59$, $P \leq 0.01$) (Appendices D-4 and D-5).

All *F. oxysporum* variables responded to *F. oxysporum* inoculum levels in test 1 (Table 4.8). The response of *F. oxysporum* variables in test 2 was less consistent than in test 1 (Table 4.9). The control treatment consistently resulted in the lowest number of ppg of soil and infection levels averaged across J2 in both tests. However, because of the long term maintenance of the experiments, the fungus contaminated the controls and established fairly high populations.

The number of *M. incognita* J2 100 $ml^{-1}$ of soil detected in test 1 was a function of an interaction between *F. oxysporum* and *M. incognita* inoculum levels ($P \leq 0.05$) (Table 4.8), but the effect was not observed in test 2 (Table 4.9). In general, the nematode J2 count remaining in the soil following harvest decreased as levels of *F. oxysporum* increased. The number of J2 100 $ml^{-1}$ of soil correlated with shoot dry weight in test 1 ($r= -0.42$, $P \leq 0.05$), and in test 2

($r = -0.59$, $P \leq 0.01$). Further, in test 1, the number of J2 correlated with root dry weight ($r = -0.40$, $P \leq 0.05$). These negative correlations are consistent with a pathogenic effect of *M. incognita* on sage.

Neither pathogen was demonstrated to be a significant factor in the die-back disease of sage. Little mortality or root weight loss occurred in these tests. However, significant effects on growth were observed and each pathogen affected sage in a different manner. *Meloidogyne incognita* alone reduced some measures of plant development and had a slight effect on survival, while *F. oxysporum* alone induced plant etiolation early in the tests and smaller main stem diameters.

Results obtained in the preliminary pathogenicity test with sage (Chapter III) provided some indication that *F. oxysporum* was pathogenic to sage; 50% and 70% mortality occurred at inoculum levels of 300 and 15,900 ppg, respectively. This response was not obtained in the combined pathogenicity tests. Since the original soil used in the preliminary test was stored for one year before being used in the combined pathogenicity study, there is the possibility that *F. oxysporum* effectiveness was lost during storage, which could explain the lack of mortality, root weight loss, or validated results between the combined pathogenicity tests.

Another factor which could have contributed to the inconsistent results was the establishment of tests in two greenhouses with different environmental conditions. Even though maximum and minimum temperatures between the glasshouses were similar, evaporative-cooled glasshouses are known to provide high relative humidity conditions, while refrigerated-air-cooled glasshouses are known to provide low relative humidity conditions. Environmental differences between the greenhouses could have influenced plant response to pathogen infection or could have altered the ability of the pathogens to infect roots and cause disease.

Due to inconsistent results between test 1 and 2, it cannot be concluded that die-back of sage results from the interaction of *F. oxysporum* and *M. incognita* race 1. The pathogens appeared to affect sage in different manners. Early in the tests (123 days after establishment), plants grown in *F. oxysporum* infested soil exhibited etiolation in response to increased plant height and internode length in both tests. Later (247 days after establishment) plant height was reduced by *M. incognita* in both tests. At harvest, plant mortality was less than 7% in both tests. Shoot or root weight loss was not confirmed between tests. Based on the inconsistent results obtained in these study, additional work should be conducted to elucidate the role of pathogens in the dieback disease.

# CHAPTER V

# COMBINED PATHOGENICITY TESTS WITH *Fusarium oxysporum* and *Meloidogyne incognita* ON LAVENDER

## Introduction

Tucker and Maciarello (1990b) reported that lavender (*Lavandula* spp.) grown under hot and humid conditions is susceptible to soilborne fungi. True lavender (*Lavandula angustifolia* Mill.) and lavandin (*Lavandula x intermedia* Emeric.) were susceptible to *Fusarium solani* Mart. in Delaware. Angell (1991) observed a high incidence of die-back disease in field-grown lavender (*Lavandula x hybrida* Balb. ex Ging.) in Florida. Lavender plants appeared stunted, with root decay and black lesions extending up the main stem from the soil line.

*Fusarium* spp. were isolated from lavender plants grown at the University of Florida from 1989 through 1990. Espaillat et al. (1993) obtained 18 isolates of *Fusarium oxysporum* Link from plants grown in a glasshouse in infested soil collected from the site where Angell (1991) made his observations. Searches of the published literature conducted by the senior author and Simon et al. (1984) failed to find previous reports of disease caused by *F. oxysporum* on lavender.

Hasseb and Pandley (1987) concluded that root-knot nematodes (*Meloidogyne* spp.) were an important constraint in the production of several medicinal and aromatic plants. Lisetskaya (1973) observed that *M. hapla* Chitwood caused moderate to severe growth reduction of lavender. Moreno et al. (1992) found lavender (*Lavandula x hybrida*) plants to have high gall ratings and egg mass indices resulting from inoculation with *M. incognita* (Kofoid and White) Chitwood race 1.

Webster (1985) documented that *F. oxysporum*, alone and in interaction with *M. incognita*, is capable of reducing plant populations and yield of many crops. No studies have been published on diseases caused by interaction of soilborne pathogens in lavender.

Powell (1971) noted that root-knot nematode damage declines due to the lack of suitable root tissue following infection by another soilborne pathogen. Bruehl (1987b) supported Powell's claim, suggesting that the organism accompanying the nematode may gain from an interaction at the expense of the nematode. Few studies have been designed with *F. oxysporum* as the primary pathogen and *M. incognita* as the secondary pathogen (Dropkin, 1980, and Mountain and McKeen, 1962). The objective of this study was to determine the roles of *F. oxysporum*, *M. incognita* race-1, and the interaction of the two organisms on growth and dieback of lavender.

## Materials and Methods

### Preparation of Inoculum

Lavender roots taken from field-grown plants exhibiting symptoms of reduced size and yellowing were plated on potato dextrose agar amended with 1.0 ml of tergitol, 100 mg of streptomycin, and 500 mg of chlortetracycline per liter of medium (Steiner and Watson, 1965). An aggressive isolate of *F. oxysporum* was identified (Nelson et al., 1983), grown on Komada's (1975) specific medium for *F. oxysporum*, and mass produced in wheat seeds (Nelson et al., 1986). Soil classified as Arredondo fine sand (loamy siliceous, hyperthermic family of Arenic and Grossarenic, Paleudults), was microwaved (Ferriss, 1984), infested with the isolate at a ratio of 10 g of inoculated wheat seeds to 1 kg of soil, and then stored in polyethylene bags in the dark at 20ºC for a month. Bags were kneaded frequently to ensure uniform colonization of the soil and to avoid clumps with a high concentration of fungus. Following storage, samples of soil from the bags were plated on Komada's (1975) medium to establish the population density, which had attained 6,900 propagules per gram (ppg) of soil of *F. oxysporum*. This population was designated the high concentration and was diluted with microwave-treated soil to create treatment levels used in the combined pathogenicity tests.

Second stage juveniles (J2) of *M. incognita* race 1 were isolated from galled roots of field grown sage plants and identified in a differential host test (Jenkins, 1964) (Chapter III). The J2 were produced in pepper (*Capsicum frutescens* L.) 'California Wonder' for 3 months, and hatched using the Baermann funnel incubation method (Rodríguez-Kábana, 1981).

Two combined pathogenicity tests were initiated in summer 1994 using $3^2$ factorial experiments in a randomized complete block design with 10 replications. The treatment factors included three inoculum levels of *M. incognita* race 1 (0, 100 and 1,000 J2 per ml) and three inoculum levels of *F. oxysporum* (0, 200 and 2,000 ppg of soil). The first pathogenicity test (test 1) was established on 29 July 1994 in an evaporative-cooled glasshouse and the second pathogenicity test (test 2) began on 13 August 1994 in a refrigerated-air-cooled glasshouse.

Healthy appearing, 1-month-old rooted cuttings of lavender were transplanted into autoclaved, 10-cm clay pots containing soil with 0, 200, or 2,000 ppg of *F. oxysporum*. Each pot was located on a water retention plate with a 10-cm spacing between pots to avoid water splash and contamination among treatments (Figure 4.1).

Lavender cuttings were rooted in a hotbed containing AERO-SOIL® horticultural perlite medium (Chemrock Corp., Grefco Inc., 3435 West Lomita Boulevard, Torrance, CA 90509). Ten days after transplant, 10 ml of a suspension of 0, 100, or 1,000 J2 of *M. incognita* race 1 per ml were injected into the appropriate treatment pot. Pots were watered as needed and drenched with Peter's® 20-20-20 liquid fertilizer every 2 weeks using 1.5 g liter$^{-1}$ of tap water.

Minimum and maximum temperatures were recorded daily and the bi-weekly mean temperature was calculated (Appendix D-3). Three spray applications of Diazinon® were required in test 2 for control of greenhouse white flies (*Trialeurodes vaporariorum* (Westwood) and cottony cushion mealy bugs (*Icerya purchasi* Maskell). Plants in test 1 were harvested 291 days after establishment, and those in test 2 were harvested after 304 days.

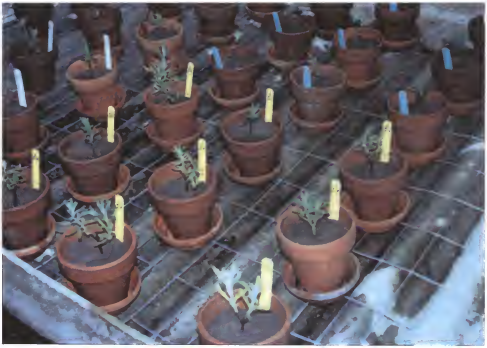

Figure 5.1. First combined pathogenicity test with lavender 3 days after establishment.

## Data collection

The percentage of dead plants and plant height were assessed for 10 replications, six times during the course of test 1 and test 2 (Appendix E-1 and E-2). Plant height was measured from the soil-line at the base of the stem to the tip of the highest leaf.

Canopy width was measured in 10 replications at 123 days after establishment in both tests, and at 247 days in test 1 and 233 days in test 2. Number of flowering plants and the number of spikes plant$^{-1}$ were counted for 10 replications at 185 and 239 days after establishment in test 1, and at 161 and at 223 days in test 2.

Internode length was calculated by dividing the length of the main stem by the number of branches. Canopy width was measured using circular templates of varying diameters; the template diameter which best fit around the plant canopy at the widest point was recorded (Appendix C-1). Stem diameter was measured at the soil-line with a caliper.

Final harvest of five replications took place at 291 days after establishment for test 1, and 304 days after establishment for test 2. The parameters measured included a final count of dead plants and plant height in both tests. Plant response data obtained from the final harvest included the following parameters: internode length, canopy width, stem diameter, and shoot and root dry weight. Shoots (all above ground vegetation) and roots were separated and dried at 70ºC for 4 days in a forced-air oven.

Pathogen and disease parameters recorded at harvest included *F. oxysporum* root rating, number of propagules of *F. oxysporum* per gram (ppg) of soil, percent *F. oxysporum* infection of main and fibrous roots, *M. incognita* gall rating, and number of *M. incognita* J2 100 $ml^{-1}$ soil.

Root necrosis and decay, resulting from *F. oxysporum*, were rated as follows on a scale of 1-6: 1 = white, healthy looking roots without adventitious roots; 2 = white, healthy looking roots with adventitious roots; 3 = white, healthy looking roots with adventitious roots and yellowish plant foliage; 4 = some brown roots with adventitious roots and yellowish and dwarf plants; 5 = many brown roots without adventitious roots, and dwarf and wilted plants; and 6 = mostly brown roots without adventitious roots, and dying plants (Appendix C-2).

The percent *F. oxysporum* infection in the main and fibrous roots was determined by plating a subsample of seven, 1.3-cm-long root pieces on Komada's (1975) medium, incubating at 27ºC with light and dark periods cycled every 12 hrs, and counting the number of infected root pieces after 7 days.

*Meloidogyne incognita* gall rating and number of *M. incognita* J2 100 $ml^{-1}$ of soil were recorded for all harvested plants. Gall ratings were recorded as follows: 0 = 0 galls, 1 = 1-2 galls, 2 = 3-10 galls, 3 = 11-30 galls, 4 = 31-100 galls, and 5 = more than 100 galls per root system per plant (Taylor and Sasser, 1978). The *M. incognita* J2 in a sample of 100 ml of soil were separated from soil using the centrifugal flotation procedure (Jenkins, 1964), and counted using a compound microscope.

### Statistical Analysis

Statistical analysis included ANOVA for a $3^2$ factorial experiment arranged in a randomized complete block design. Simple linear correlations between all variables were calculated for all harvest data. Logarithmic transformation [Log(x+1)] was done on the following data: number of *F. oxysporum* ppg of soil and number of *M. incognita* J2 100 $ml^{-1}$ of soil obtained at harvest from both tests. The Michigan State University Statistical Program (MSTAT-C, 1992) was used to analyze all data.

## Results and Discussion

### Measurements over Time

The effect of the two pathogens on percent dead plants was not consistent between test 1 and 2. In test 1, there was no detectable effect of the pathogens on percent dead plants (Table 5.1). In test 2, a consistent effect of *F. oxysporum* on percent dead plants occurred during the last 200 days of the study (Table 5.2). At harvest, plant mortality decreased in plants inoculated with *F. oxysporum* ($P \leq 0.01$), however, mortality rates were low overall (Table 5.2). This response agrees with results obtained in the preliminary pathogenicity test, where mortality of lavender plants was not affected by *F. oxysporum* (Chapter III).

Pathogen effect on plant height over time was inconsistent between test 1 and test 2 (Tables 5.3 and 5.4). In test 2, *F. oxysporum* increased plant height at all dates, except the first (Tables 5.4). In test 1, *M. incognita* averaged across *F. oxysporum* levels, reduced plant height on the last sampling date ($P \leq 0.01$) (Table 5.3). No interaction effect between pathogens was detected in either test for percent dead plants or plant height of lavender.

## Non-destructive Sample

At 123 days after establishment, canopy width was consistently reduced by increasing levels of *F. oxysporum* inoculum in test 1 ($P \leq 0.10$) (Table 5.5) and test 2 ($P \leq 0.01$) (Table 5.6). At the same time, plant height was not affected by *F. oxysporum* inoculum levels in test 1 (Table 5.3). Plant height averaged across *M. incognita* levels at 123 days in test 2 increased to a high at 2,000 ppg of soil and a low at 200 ppg of soil, which corresponded with the largest canopy width at 2,000 ppg of soil and the smallest canopy width at 200 ppg of soil (Table 5.4). Although not validated by test 1, the data in test 2 indicate that

Table 5.1. Means and ANOVA summary of the effects of *Fusarium oxysporum* and *Meloidogyne incognita* inoculum levels on the percent dead plants in the first pathogenicity test with lavender[†].

| Pathogens | | Days after establishment | | | | | |
|---|---|---|---|---|---|---|---|
| *Fusarium oxysporum* | *Meloidogyne incognita* | 48 | 72 | 101 | 170 | 254 | 291 |
| ppg[§] | J2 | ------------- | percent dead plants[‡] | | | | -------------- |
| 0 | 0 | 0 | 0 | 1 | 2 | 4 | 5 |
| 0 | 100 | 0 | 0 | 0 | 1 | 2 | 2 |
| 0 | 1000 | 0 | 0 | 0 | 1 | 1 | 1 |
| 200 | 0 | 1 | 1 | 1 | 1 | 2 | 3 |
| 200 | 100 | 0 | 0 | 0 | 2 | 3 | 4 |
| 200 | 1000 | 0 | 1 | 1 | 3 | 3 | 5 |
| 2000 | 0 | 2 | 2 | 2 | 3 | 5 | 5 |
| 2000 | 100 | 1 | 0 | 1 | 1 | 2 | 3 |
| 2000 | 1000 | 0 | 0 | 1 | 2 | 3 | 4 |
| ANOVA: | | | | | | | |
| Fusarium (A) | | n s[¶] | n s | n s | n s | n s | n s |
| Meloidogyne (B) | | n s | n s | n s | n s | n s | n s |
| Interaction (AXB) | | n s | n s | n s | n s | n s | n s |
| CV (%) | | 469 | 381 | 353 | 209 | 162 | 130 |

[†] Data at all dates analyzed with ANOVA as a $3^2$ Factorial.

[‡] Percent dead plants at specific dates after planting 10 plants per treatment.

[§] ppg = propagules per gram of soil, and J2 = juveniles 100 $ml^{-1}$ of soil.

[¶] n s = F value non significant.

Table 5.2. Means and ANOVA summary of the effects of *Fusarium oxysporum* and *Meloidogyne incognita* inoculum levels on the percent dead plants in the second pathogenicity test with lavender[†].

| Pathogens | | Days after establishment | | | | | |
|---|---|---|---|---|---|---|---|
| *Fusarium oxysporum* | *Meloidogyne incognita* | 48 | 72 | 101 | 170 | 238 | 304 |
| ppg[§] | J2 | percent dead plants[‡] | | | | | |
| 0 | 0 | 0 | 2 | 4 | 5 | 6 | 6 |
| 0 | 100 | 1 | 1 | 2 | 2 | 3 | 4 |
| 0 | 1000 | 3 | 3 | 4 | 6 | 6 | 7 |
| 200 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| 200 | 100 | 1 | 1 | 1 | 3 | 3 | 3 |
| 200 | 1000 | 1 | 1 | 1 | 2 | 2 | 2 |
| 2000 | 0 | 0 | 0 | 0 | 0 | 1 | 2 |
| 2000 | 100 | 0 | 0 | 1 | 2 | 3 | 3 |
| 2000 | 1000 | 0 | 1 | 2 | 3 | 3 | 3 |
| ANOVA: | | | | | | | |
| Fusarium (A) | | n s[¶] | n s | ¥ | ¥ | * * | * * |
| Meloidogyne (B) | | n s | n s | n s | n s | n s | n s |
| Interaction (AXB) | | n s | n s | n s | n s | n s | n s |
| CV (%) | | 367 | 248 | 162 | 175 | 144 | 136 |

[†] Data at all dates analyzed with ANOVA as a $3^2$ Factorial.

[‡] Percent dead plants at specific dates after planting 10 plants per treatment.

[§] ppg = propagules per gram of soil, and J2 = juveniles 100 $ml^{-1}$ of soil .

[¶] n s = F value non significant; ** = F value significant at $P \leq 0.01$;
¥ = F value significant at $P \leq 0.10$.

Table 5.3. Means and ANOVA summary of the effects of *Fusarium oxysporum* and *Meloidogyne incognita* inoculum levels on plant height in the first pathogenicity test with lavender.[†]

| Pathogens | | Days after establishment | | | | | |
|---|---|---|---|---|---|---|---|
| *Fusarium oxysporum* | *Meloidogyne incognita* | 2 | 45 | 123 | 239 | 247 | 294 |
| ppg[§] | J2 | plant height (cm)[‡] | | | | | |
| 0 | 0 | 9.2 | 9.6 | 14.7 | 21.9 | 27.4 | 53.9 |
| 0 | 100 | 9.4 | 11.1 | 16.2 | 24.7 | 34.2 | 54.2 |
| 0 | 1000 | 9.0 | 8.6 | 11.8 | 21.9 | 32.2 | 47.0 |
| 200 | 0 | 7.7 | 8.3 | 23.3 | 33.2 | 33.1 | 55.1 |
| 200 | 100 | 7.7 | 9.3 | 16.1 | 20.5 | 26.1 | 48.9 |
| 200 | 1000 | 7.6 | 7.8 | 13.3 | 18.1 | 20.5 | 41.5 |
| 2000 | 0 | 8.5 | 8.1 | 13.4 | 24.3 | 23.4 | 53.6 |
| 2000 | 100 | 8.2 | 7.9 | 13.6 | 25.2 | 33.6 | 53.8 |
| 2000 | 1000 | 7.4 | 7.6 | 14.0 | 20.8 | 21.6 | 46.8 |
| ANOVA: | | | | | | | |
| Fusarium (A) | | n s[¶] | ¥ | n s | n s | n s | n s |
| Meloidogyne (B) | | n s | n s | n s | n s | n s | * * |
| Interaction (AXB) | | n s | n s | n s | n s | n s | n s |
| CV (%) | | 18 | 37 | 68 | 65 | 70 | 17 |

[†] Data at all dates analyzed with ANOVA as a $3^2$ Factorial.

[‡] Cumulative plant height measured from the soil line to the tip of the highest leaf taken at specific dates after establishment of the experiment.

[§] ppg = propagules per gram of soil, and J2 = juveniles 100 $ml^{-1}$ of soil.

[¶] n s = F value non significant; ** = F value significant at $P \leq 0.01$; ¥ = F value significant at $P \leq 0.10$.

Table 5.4. Means and ANOVA summary of the effects of *Fusarium oxysporum* and *Meloidogyne incognita* inoculum levels on plant height in the second pathogenicity test with lavender.[†]

| Pathogens | | Days after establishment | | | | | |
|---|---|---|---|---|---|---|---|
| *Fusarium* oxysporum | *Meloidogyne* incognita | 2 | 45 | 123 | 239 | 247 | 304 |
| ppg[§] | J2 | plant height[‡] | | | | | |
| 0 | 0 | 10.0 | 9.4 | 17.9 | 14.9 | 20.5 | 31.2 |
| 0 | 100 | 9.1 | 7.3 | 14.7 | 20.3 | 28.2 | 45.2 |
| 0 | 1000 | 9.8 | 7.6 | 13.1 | 15.9 | 25.4 | 43.4 |
| 200 | 0 | 7.9 | 6.5 | 14.7 | 29.0 | 34.3 | 43.8 |
| 200 | 100 | 7.9 | 6.4 | 13.7 | 27.2 | 33.9 | 48.4 |
| 200 | 1000 | 8.8 | 5.2 | 12.7 | 28.0 | 35.1 | 48.4 |
| 2000 | 0 | 7.6 | 7.9 | 18.2 | 34.0 | 40.5 | 51.6 |
| 2000 | 100 | 8.2 | 8.3 | 17.8 | 25.5 | 34.7 | 52.6 |
| 2000 | 1000 | 7.3 | 8.0 | 16.6 | 22.1 | 31.0 | 48. |
| ANOVA: | | | | | | | |
| Fusarium (A) | | n s[¶] | * * | * | * | * | * |
| Meloidogyne (B) | | n s | n s | n s | n s | n s | n s |
| Interaction (AXB) | | n s | n s | n s | n s | n s | n s |
| CV (%) | | 24 | 51 | 36 | 77 | 53 | 28 |

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[†] Data at all dates analyzed with ANOVA as a $3^2$ Factorial.

[‡] Cumulative plant height measured from the soil line to the tip of the highest leaf taken at specific dates after establishment of the experiment.

[§] ppg = propagules per gram of soil, and J2 = juveniles 100 $ml^{-1}$ of soil.

[¶] n s = F value non significant; ** = F value significant at $P \leq 0.01$;
* = F value significant at $P \leq 0.05$.

Table 5.5. Treatment means and analysis of variance summary for additional agronomic data collected at different dates after establishment of the first pathogenicity test with lavender[†].

| Pathogens | | Agronomic variables[‡] | | | | Number |
|---|---|---|---|---|---|---|
| *Fusarium* | *Meloidogyne* | Canopy width[§] | | Flowering | | Spikes at |
| *oxysporum* | *incognita* | 123 | 247 | 185 | 239 | 239 days |
| ppg[¶] | J2 | ---------- cm ---------- | | ---------- number | ---------- | ---------- |
| 0 | 0 | 15.8 | 16.4 | 0.3 | 0.6 | 5.4 |
| 0 | 100 | 18.4 | 19.8 | 0.3 | 0.8 | 6.9 |
| 0 | 1000 | 17.2 | 21.5 | 0 | 0.9 | 6.8 |
| 200 | 0 | 15.5 | 18.8 | 0.2 | 0.6 | 4.8 |
| 200 | 100 | 16.1 | 14.3 | 0.2 | 0.6 | 3.2 |
| 200 | 1000 | 12.8 | 14.7 | 0 | 0.4 | 2.6 |
| 2000 | 0 | 12.9 | 13.1 | 0.1 | 0.5 | 5.1 |
| 2000 | 100 | 14.2 | 19.5 | 0 | 0.7 | 5.8 |
| 2000 | 1000 | 15.5 | 14.4 | 0.1 | 0.6 | 2.6 |
| ANOVA: | | | | | | |
| Fusarium (A) | | ¥[#] | n s | n s | n s | ¥ |
| Meloidogyne (B) | | n s | n s | n s | n s | n s |
| Interaction (AXB) | | n s | n s | n s | n s | n s |
| CV (%) | | 34 | 66 | 245 | 72 | 99 |

[†] All variables were analyzed for ANOVA as a $3^2$ Factorial.

[‡] Agronomic variables: canopy width at 123 and 247 days after establishment; flowering counts the number of flowered plants at 185 and 239 days; and number of spikes per plant at 239 days.

[§] days = days after experiment establishment.

[¶] ppg = number of propagules per gram of soil, and J2 = number of juveniles 100 $ml^{-1}$ of soil.

[#] n s = F value non significant; ¥ = F value significant at $P \leq 0.10$.

Table 5.6. Treatment means and analysis of variance summary for additional agronomic data collected at different dates after establishment of the second pathogenicity test with lavender[†].

| Pathogens | | Agronomic variables[‡] | | | | Number |
|---|---|---|---|---|---|---|
| *Fusarium* | *Meloidogyne* | Canopy width | | Flowering | | Spikes at |
| *oxysporum* | *incognita* | 123 days | 233 days | 161 days | 223 days | 223 days |
| ppg[§] | J2 | --------- cm | --------- | ------------- | number | --------------- |
| 0 | 0 | 23.9 | 14.1 | 0.4 | 0.4 | 8.5 |
| 0 | 100 | 21.1 | 13.9 | 0.2 | 0.6 | 9.5 |
| 0 | 1000 | 18.5 | 16.9 | 0.2 | 0.5 | 7.1 |
| 200 | 0 | 18.0 | 18.6 | 0.2 | 0.8 | 8.6 |
| 200 | 100 | 15.0 | 20.2 | 0.4 | 0.7 | 6.1 |
| 200 | 1000 | 16.9 | 18.7 | 0 | 0.8 | 9.3 |
| 2000 | 0 | 21.5 | 26.3 | 0.1 | 0.9 | 13.3 |
| 2000 | 100 | 22.5 | 19.2 | 0.2 | 0.7 | 10.6 |
| 2000 | 1000 | 19.6 | 15.2 | 0 | 0.5 | 5.3 |
| ANOVA: | | | | | | |
| Fusarium (A) | | * *[¶] | n s | n s | ¥ | n s |
| Meloidogyne (B) | | n s | n s | n s | n s | n s |
| Interaction (AXB) | | n s | n s | n s | n s | n s |
| CV (%) | | 35 | 77 | 207 | 74 | 100 |

[†] All variables were analyzed for ANOVA as a $3^2$ Factorial.

[‡] Agronomic variables: canopy width = at 123 and 233 days after establishment; flowering = the number of flowered plants at 161 and 223 days; and number of spikes per plant at 239 days after establishment.

[§] ppg = number of propagules per gram of soil, and J2 = number of juveniles 100 $ml^{-1}$ of soil.

[¶] n s = F value non significant; ** = F value significant at $P \leq 0.01$;
¥ = F value significant at $P \leq 0.10$.

*F. oxysporum* may stimulate plant height and canopy width.

The number of flowering plants was not affected by either pathogen in test 1, while number of spikes was reduced by *F. oxysporum* ($P \leq 0.10$) (Table 5.5). In test 2, an increase in *F. oxysporum* inoculum level resulted in a slight increase in number of flowering plants at 223 days after establishment ($P \leq 0.10$). However, this response was not validated by test 1.

## Harvest Data

No pathogen effect was detected at harvest on stem diameter for either test (Table 5.7). Canopy width data were not consistent between tests (Tables 5.7 and 5.8).

Effect of pathogens on shoot dry weight and root dry weight data in test 1 was not consistent with weight data in test 2 (tables 5.7 and 5.8). In test 1, both variables were reduced as *M. incognita* inoculum level increased ($P \leq 0.05$), but in test 2, shoot dry weight was not affected by pathogen levels.

Root dry weight was reduced by increasing levels of *M. incognita* in test 1 ($P \leq 0.05$) (Table 5.7), but not in test 2 (Table 5.8). Root dry weight correlated with different variables in both tests. In test 1, root dry weight correlated with *F. oxysporum* inoculum levels ($r = -0.40$, $P \leq 0.05$) and root rating ($r = -0.57$, $P \leq 0.01$), which was

Table 5.7. Summary of treatment means and analysis of variance for agronomic, *Fusarium oxysporum* and *Meloidogyne incognita* variables in the first pathogenicity test with lavender at harvest[†].

| Pathogens | | Agronomic variables[‡] | | | | *F. oxysporum* variables[$] | | | | | *M.incognita* variables[¶] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *F.oxy-sporum* | *M.incog-nita* | Stem Diam | Canopy Width | Dry Weight Shoot | Dry Weight Root | Root Rating | ppg soil Amount | ppg soil Log | Root infection main | Root infection fibrous | Gall Rating | J2 in soil number | J2 in soil log |
| ppg[#] | J2 | -mm- | - cm - | --- g --- | | | - no.- | | ---- % ----- | | | | |
| 0 | 0 | 7.2 | 25.0 | 29.5 | 34.1 | 1.0 | 3476 | 1.8 | 3.3 | 13.3 | 0 | 747 | 2.7 |
| 0 | 100 | 6.2 | 24.9 | 28.6 | 32.4 | 3.0 | 7467 | 3.6 | 5.0 | 12.5 | 0 | 101 | 1.9 |
| 0 | 1000 | 6.3 | 23.2 | 25.8 | 24.9 | 1.3 | 5267 | 3.4 | 36.7 | 35.0 | 0 | 538 | 2.4 |
| 200 | 0 | 7.7 | 26.6 | 29.0 | 24.8 | 2.3 | 49067 | 4.5 | 93.3 | 100.0 | 0 | 73 | 0.8 |
| 200 | 100 | 7.5 | 22.5 | 26.1 | 24.8 | 3.7 | 32333 | 4.5 | 46.7 | 100.0 | 0.2 | 2554 | 2.4 |
| 200 | 1000 | 6.4 | 22.4 | 24.4 | 21.0 | 3.3 | 21000 | 4.2 | 63.3 | 76.7 | 0 | 889 | 1.9 |
| 2000 | 0 | 6.5 | 27.4 | 28.6 | 23.0 | 3.7 | 36666 | 4.3 | 96.7 | 100.0 | 0 | 838 | 1.1 |
| 2000 | 100 | 6.7 | 23.4 | 27.3 | 24.0 | 3.6 | 81000 | 4.9 | 86.7 | 100.0 | 0 | 435 | 1.9 |
| 2000 | 1000 | 6.8 | 23.4 | 24.4 | 21.8 | 3.3 | 43400 | 4.6 | 100.0 | 100.0 | 0.6 | 4231 | 3.6 |
| ANOVA: | | | | | | | | | | | | | |
| Fusarium (A) | | n s[††] | n s | n s | n s | n s | * * | * * | * * | * * | n s | ¥ | n s |
| Meloidogyne (B) | | n s | ¥ | ¥ | * | n s | n s | n s | n s | n s | * * | ¥ | n s |
| Interact. (AXB) | | n s | n s | n s | n s | n s | n s | n s | n s | n s | n s | * * | n s |
| CV (%) | | 12 | 17 | 15 | 31 | 57 | 88 | 23 | 53 | 28 | 510 | 98 | 55 |

[†] All variables analyzed with ANOVA of a $3^2$ Factorial.

[‡] Agronomic variables: stem diameter measured at the soil line; dry weights after 3 days at 70ºC.

[$] *Fusarium oxysporum* variables: root rating using a scale of 1-6; colony forming units in the soil at harvest; logarithm (x+1) of number ppg in soil, and percent of main and fibrous roots infected.

[¶] *Meloidogyne incognita* variables: gall rating using the scale of 0-5; number of J2 of *M.incognita* race 1 in 100 $ml^{-1}$ of soil; Log J2 in soil = logarithm (x+1) of number J2 in soil.

[#] ppg = propagules per gram of soil, and J2 = juveniles 100 $ml^{-1}$ of soil.

[††] n s = F value non significant; ** = F value significant at $P<0.01$; * = F value significant at $P<0.05$; ¥ = F value significant at $P<0.10$.

Table 5.8. Summary of treatment means and analysis of variance for agronomic, *Fusarium oxysporum* and *Meloidogyne incognita* variables in the second pathogenicity test with lavender at harvest[†].

| Pathogens | | Agronomic variables[‡] | | | | *F. oxysporum* variables[§] | | | | | *M. incognita* variables[¶] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *F. oxysporum* | *M. incognita* | Stem | Canopy | Dry Weight | | Root | ppg in soil | | Root infection | | Gall | J2 in soil | |
| | | Diam | Width | Shoot | Root | Rating | Amount | log | main | fibrous | Rating | number | log |
| ppg[#] | J2 | -mm- | - cm - | --- g --- | | | -no.- | | ---- % ----- | | | | |
| 0 | 0 | 5.7 | 19.0 | 25.1 | 16.3 | 2.0 | 21800 | 4.3 | 21.7 | 61.7 | 0 | 285 | 2.4 |
| 0 | 100 | 7.5 | 28.7 | 31.9 | 20.3 | 2.3 | 26600 | 4.3 | 30.0 | 75.0 | 0 | 1347 | 2.2 |
| 0 | 1000 | 7.2 | 29.6 | 29.3 | 26.0 | 1.3 | 17933 | 3.8 | 45.0 | 73.3 | 1.0 | 161 | 1.3 |
| 200 | 0 | 6.1 | 24.3 | 25.2 | 18.3 | 2.0 | 7933 | 3.9 | 75.0 | 98.3 | 0 | 413 | 2.3 |
| 200 | 100 | 6.7 | 25.8 | 27.0 | 16.3 | 3.7 | 45267 | 4.6 | 96.7 | 99.2 | 0 | 674 | 2.5 |
| 200 | 1000 | 7.1 | 28.5 | 29.8 | 20.8 | 4.0 | 81267 | 4.8 | 71.7 | 95.8 | 0 | 348 | 2.0 |
| 2000 | 0 | 7.0 | 30.3 | 30.4 | 23.2 | 1.7 | 16067 | 4.2 | 90.0 | 100.0 | 0 | 139 | 1.3 |
| 2000 | 100 | 6.4 | 31.4 | 31.1 | 22.7 | 2.7 | 26667 | 4.4 | 91.7 | 90.0 | 0 | 194 | 1.6 |
| 2000 | 1000 | 7.5 | 26.4 | 30.1 | 25.1 | 3.3 | 41333 | 4.6 | 88.3 | 100.0 | 0.8 | 57 | 1.3 |
| ANOVA: | | | | | | | | | | | | | |
| Fusarium (A) | | n s[††] | n s | n s | n s | n s | * * | n s | * * | * * | n s | n s | n s |
| Meloidogyne (B) | | n s | n s | n s | n s | n s | ¥ | n s | n s | n s | n s | n s | n s |
| Interact. (AXB) | | n s | n s | n s | n s | n s | n s | ¥ | n s | n s | n s | n s | n s |
| CV (%) | | 25 | 26 | 22 | 37 | 48 | 85 | 9 | 43 | 15 | 484 | 221 | 57 |

† All variables analyzed with ANOVA of a $3^2$ Factorial.

‡ Agronomic variables: stem diameter measured at the soil-line; dry weights after 3 days at 70ºC.

§ *Fusarium oxysporum* variables: root rating using a scale of 1-6; colony forming units in the soil at harvest; logarithm (x+1) of number ppg in soil; and percent of main and fibrous roots infected.

¶ *Meloidogyne incognita* variables: gall rating based on a scale of 0-5; number of J2 of *M.incognita* race 1 in 100 ml of soil; Log J2 in soil = logarithm (x+1) of number J2 in soil.

# ppg = propagules per gram of soil, and J2 = juveniles 100 $ml^{-1}$ of soil .

†† n s = F value non significant; ** = F value significant at $P<0.01$; * = F value significant at $P<0.05$; ¥ = F value significant at $P<0.10$.

consistent with pathogenicity of *F. oxysporum* to lavender (Appendix E-3).

All *F. oxysporum* variables in test 1, except root rating, responded to *F. oxysporum* inoculum levels ($P \leq 0.01$) (Table 5.7). The lowest infection values were consistently obtained in the control for both tests. Confirmation of infection in both tests is an important validation of the methodology employed, but little disease occurred after infection.

*Meloidogyne incognita* variables responded more in test 1 than in test 2. There was limited gall formation in test 1, gall rating increased as the nematode inoculum levels increased ($P \leq 0.01$) (Table 5.7). The average number of J2 100 $ml^{-1}$ of soil in test 1 increased with increased *F. oxysporum* inoculum levels ($P \leq 0.10$), and with increased levels of *M. incognita* ($P \leq 0.10$). An interaction effect of the two pathogens ($P \leq 0.01$) demonstrated that soil nematode population increased in the presence of both pathogens, with the combined high inoculum level of both pathogens, resulting in the highest number of J2. However, the control was contaminated and the response was not validated by the results of test 2.

Despite the extended duration of both tests there was little confirmation of results between test 1 and test 2. Therefore, it cannot be concluded that die-back of lavender results from infection by *F. oxysporum* or *M. incognita* race

1 or from the interaction of the two organisms. Mortality was less than 7% in both tests. In test 2, *F. oxysporum* reduced plant mortality and stimulated plant etiolation and flowering. Shoot and root weight loss was not confirmed between tests.

The low level of gall formation in lavender roots is a strong indication of resistance to *M. incognita*. Lavender roots contain the terpenoid linalool, considered by Hasseb et al. (1978) and Chatterjee et al.(1982) to have nematicidal properties. Based on the inconsistent results obtained in this study, additional work should be conducted to elucidate the roles of potential pathogens in dieback of lavender.

## CHAPTER VI

## DISEASE CONTROL STUDY WITH SAGE

### Introduction

Sage (*Salvia officinalis* L.) is a hardy, perennial evergreen shrub native to the Dalmatian coast of Yugoslavia near the Adriatic sea. Duke and Hurst (1975), using the Holdridge (1966) life zone system, considered sage to be adapted for growth from the Cool Temperate Moist Forest life zone (6-12ºC; 500-1,000 mm of rain $yr^{-1}$) to the Tropical Dry Forest life zone (>24ºC; 1,000-2,000 mm of rain $yr^{-1}$).

De Baggio (1987) stated that sage tolerates a range of soil pH from 5.3 to 8.3, but grows best in alkaline soils. Robbins (1988) reported that sage grows and yields better with an application of 13 to 17 metric tons of dolomitic limestone $ha^{-1}$ $year^{-1}$. Engelhard and Wolz (1973) found that raising soil pH to 7.0 reduced the level of *Fusarium* wilt in Chrysanthemum (*Chrysanthemum* spp.). Bruehl (1987) warned that it is not always practical to control soilborne diseases by modifying soil pH alone.

Tucker and Maciarello (1990b) observed that, under hot and humid conditions, sage becomes susceptible to soilborne fungi. Angell (1991) observed a high incidence of a die-back disease of sage at the Green Acres Agronomy Farm, Gainesville, FL. Sage plants appeared stunted with root decay and black lesions extended up the main stem from the soil line. Espaillat et al. (1993) obtained 18 isolates of *Fusarium oxysporum* Link from plants grown in a glasshouse in infested soil collected from the site where Angell (1991) made his observations. A search of the published literature conducted by the senior author, as well as a search by Simon et al. (1984), failed to find previous reports of disease caused by *F. oxysporum* on sage.

Hasseb and Pandley (1987) concluded that root-knot nematodes (*Meloidogyne* spp.) are an important constraint in the production of several medicinal and aromatic plants. Lisetskaya (1973) diagnosed *M. hapla* Chitwood in roots of sage. Hibrahim et al. (1983), in a nematode screening test, found sage to be susceptible to *M. arenaria* Neal race 1, *M. incognita* Kofoid and White races 1 and 2, and *M. javanica* Treud. Moreno et al. (1992) found sage plants to have high gall and egg mass indices resulting from inoculation with *M. incognita* race 1.

Balbaa (1983) and Latin (1986) found a scarcity of specific information on herb production techniques and disease control, including few registered fungicides. An index of scientific publications on herbs, aromatic and medicinal plants compiled by Simon et al. (1984) included a section in plant protection and disease control. Out of 239 publications listed, only 99 publications (41%) dealt with general disease control measures, but none were on sage. On a world scale, there is a lack of scientific literature on the use of cultural practices on herbs. This is especially important when considering that Morris and Cracker (1990), and herb producers in general, associate herb production with the organic farming philosophy.

Trivedi and Barker (1986) suggested the development of integrated disease management strategies which include cultural practices. Kharbanda and Tewari (1996) considered that most cultural practices aim at reducing the amount of primary inoculum in the soil. Palti (1981) estimated that, through suitable adjustments and combinations of cultural practices, it is possible to modify the environment so that conditions become unfavorable for one or more pathogens and subsequent disease development.

Rich (1986) found the suppression of soilborne fungi and nematodes to be important benefits of mulching, which improves plant development and yield as a result of nutrient release and changes in soil microclimate. Aust et al. (1980) and Huber and Watson (1970) determined that organic mulches raise the "biological buffering capacity" by enriching the soil with microflora competitive with or antagonistic to pathogens.

To develop a better understanding of die-back disease of field grown sage, and to provide herb growers in North-Central Florida with information on alternative disease control methods, the following objectives were established: 1) assess the die-back disease of sage over time; 2) evaluate the effect of soil management practices on growth and survival of sage; and 3) evaluate the effect of manipulation of soil microclimate with applications of methyl bromide, lime, organic mulch and synthetic mulch for the control of *Fusarium oxysporum* Link and *Meloidogyne incognita* Kofoid and White race 1.

## Materials and Methods

A field disease control study was conducted from April 1992 to August 1993 at the Green Acres Agronomy Farm, 22 km west of Gainesville, Florida. The soil at the experimental site was classified as Arredondo fine sand (loamy siliceous, hyperthermic family of Arenic and Grossarenic Paleudults). Angell (1991) reported a high incidence of a die-back disease in sage grown at the site in previous research (Figure 6.1).

The experimental area was kept under annual ryegrass (*Lolium multiflorum* Lam.) during the winter and fallow during the summer for 2 years preceding establishment of the disease control study. In February 1992, the study area was plowed, followed by a disc harrow.

Experimental Design

The experiment was established in April 1992 using a randomized complete block design arranged in split-plots with four replications. Main plots consisted of three soil amendment treatments: methyl bromide, lime, and nonamended. Subplots were three mulch treatments: organic mulch, synthetic mulch, and nonmulched (Figure 6.2). The subplots were 2.75 $m^2$ and contained 20 plants arranged in four rows with five plants per row. Rows were spaced 0.45 m apart with 0.30 m between plants in a row.

Methyl bromide (Brom-o-gas® = 98% methyl bromide plus 2% chloropicrin) was applied at a rate of 1.1 kg in 9.18 $m^{-2}$. Prior to methyl bromide application, the area was irrigated with 50 mm of water. Methyl bromide was applied in a chamber constructed with polyethylene sheets over the plot area. After 2 days of treatment, the polyethylene sheets were removed, allowing the soil to aerate for 15 days prior to transplant.

Lime treatment plots were maintained at pH ± 7.2. Adjustment of soil pH was based on calculations using the method of Adams and Evans (1962) and average plot soil pH. Pulverized dolomitic limestone was applied uniformly across the whole plot. Five applications of 5,570 kg $ha^{-1}$ (5.5 metric tons) each were made to raise and maintain soil pH

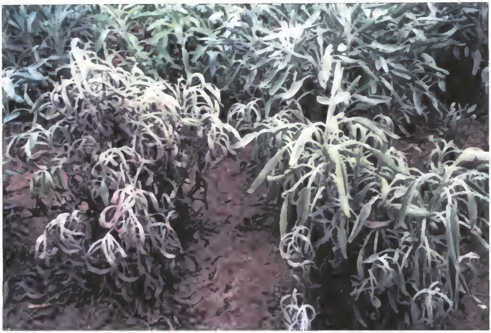


Figure 6.1. Diseased sage plants grown at the Green Acres Agronomy farm, Gainesville, Florida.

Figure 6.2. General view of the disease control study with sage in August 1992, 4 months after establishment.

close to 7.2 (Appendix C-4). The first lime application was made 2 months before transplant. The need for subsequent lime applications was determined by soil pH monitoring. Nonamended plots were maintained with a bare soil surface.

Dry, shredded perennial peanut (*Arachis glabrata* Benth) hay, fumigated with methyl bromide at a rate of 1 kg fumigant to 4 $m^3$ served as the organic mulch. The first organic mulch application of 3,300 kg $ha^{-1}$ was made after sage transplant, forming a ground cover 3 cm thick. Four subsequent applications of 2,200 kg $ha^{-1}$ each (totalling 12.2 metric tons of hay mulch $ha^{-1}$ $year^{-1}$) were applied as needed on top of the previous decomposing hay mulch to maintain a constant organic mulch cover.

The synthetic mulch treatment was "pro5 Weed Barrier"® (DeWitt company, Sikeston, Missouri), a black and woven polypropylene ground cover which stops weeds and allows water and nutrients to pass through. Because of the porous nature of Pro5, the use of overhead irrigation and over the top applications of lime, fertilizers and fungicides were possible.

Synthetic mulch material was cut into 1.9- X 2.1-m pieces to fit over the appropriate treatment plots. Holes (12-cm diameter) were melted in the Pro5 material with a propane torch to accommodate a transplant. The synthetic mulch was placed over the plot area and secured with loose soil along the perimeter edges of the mulch.

Nonmulched treatment plots were maintained free of weeds and other organic residues. The nonamended plus nonmulched treatment combination served as the control for analysis of response variables.

In June 1992, healthy appearing, 1-month-old rooted cuttings of sage were transplanted. Overhead sprinkler irrigation was provided as needed. Fertilizer was applied every 8 weeks in accordance with Angell's (1991) findings of 170 kg of nitrogen and 80 kg of potassium $ha^{-1}$ $year^{-1}$ for sage grown at the same site. The total amount of fertilizer applied $year^{-1}$ was split into six applications, the first after transplant, with subsequent applications every 2 months. Pure sand was mixed with the fertilizer, at a proportion of 2:1, to facilitate distribution over the entire plot.

Plots were kept weed free by directed applications of Roundup (Glyphosate) as often as required at a dosage of 13 ml $liter^{-1}$ of tap water, plus 13 ml non-ionic surfactant $liter^{-1}$ of tap water. Herbicide application was made using a 7.5-liter $CO_2$ powered sprayer. Roundup was applied eight times, six times during the first year and two times during the first 6 months of the second year. Between herbicide applications weeds were removed by hand and a hoe.

## Data Collection

Following application of soil amendment treatments (August 1992), population densities of *F. oxysporum* were determined for each plot by plating soil samples in Komada's (1975) specific medium for *F. oxysporum*. After 7 days of incubation at 27ºC with a cycle of 12 hours of light and 12 hours of darkness, the numbers of *F. oxysporum* ppg of soil were counted (Appendix B-2).

Parameters recorded periodically during the experiment included plant height (mean of four plants per plot), percent dead plants, and soil temperature at 5- and 10-cm depths. In September 1993, 1.5 years after establishment, data collection was stopped due to severe plant mortality, and the remaining plants were harvested. Parameters recorded at harvest included shoot dry weight, root dry weight, *M. incognita* gall rating, *F. oxysporum* root rating, percent root infection by *F. oxysporum* and population density of both *F. oxysporum* and *M. incognita*.

Plant height data were collected in cm using a 2-m metallic ruler. The measurements were taken from the soil-line to the tip of the highest leaf. Soil temperature was measured using a digital electronic recording device with a soil probe placed at 5- and 10-cm depths.

At harvest (September 1993) whole plants were removed from soil, washed, and evaluated for infection by *F.*

*oxysporum* and *M. incognita*. Soil was sampled from around plant roots for evaluation of pathogen population density.

Roots were rated for *M. incognita* gall formation based on a 0-5 rating scale, where 0 = 0 galls, 1 = 1-2 galls, 2 = 3-10 galls, 3 = 11-30 galls, 4 = 31-100 galls, and 5 = more than 100 galls per plant root system (Taylor and Sasser, 1978) (Appendix C-3). Roots were rated for necrosis and decay caused by *F. oxysporum* on a scale of 1-6, where 1 = white, healthy looking roots without adventitious roots; 2 = white, healthy looking roots with adventitious roots; 3 = white, healthy looking roots with adventitious roots and yellowish plant foliage; 4 = some brown roots with adventitious roots and yellowish and dwarf plants; 5 = many brown roots without adventitious roots, and dwarf and wilted plants; and 6 = mostly brown roots without adventitious roots, and dying plants (Appendix C-2).

Population density of *F. oxysporum* in the soil was determined by plating a soil sample in Komada's (1975) medium, incubating at 27ºC with light and dark cycled every 12 hours, and counting the number of ppg of soil after 7 days. Population density of *M. incognita* 100 $ml^{-1}$ of soil was obtained by centrifugal flotation (Jenkins, 1964) (Appendix B-5). Subsamples of galled roots were incubated in Baermann funnels to hatch nematode eggs (Rodríguez-Kábana, 1981). Hatched juveniles (J2) were used in a differential host test to establish species and race of root-knot nematode forming galls on sage roots (Taylor and Sasser, 1978). Sage shoots and roots were separated and dried at 70ºC for 4 days in an forced-air oven and weighed.

### Statistical Analysis

Data were analyzed using analysis of variance (ANOVA) for a randomized complete block design arranged in split-plots. *Meloidogyne incognita* gall rating, shoot dry weight, and root dry weight were analyzed using ANOVA for a completely randomized design. Split-plot design was eliminated from the analysis of harvest data due to plant loss in the nonamended plus nonmulched treatment combination. Single degree of freedom orthogonal contrasts were done for gall rating for separation of treatment differences. Population densities of *F. oxysporum* (ppg of soil) and *M. incognita* (J2 100 $ml^{-1}$ of soil) were analyzed using ANOVA for a randomized, complete block design arranged in split-plots with two replications. All data were analyzed using MSTAT-C (1992) statistical program.

## Results and Discussion

Soil population density of *F. oxysporum* 2 months after application of methyl bromide in treatment plots was 280 ppg of soil. *Fusarium oxysporum* population densities found in lime treated plots (1,480 ppg) and nonamended plots (1,760 ppg) demonstrate the effectiveness of methyl bromide in reducing population of the pathogen (Appendix C-4).

Plant height was affected by soil amendment treatments from September 1992 to June 1993 (Table 6.1; Figure 6.3). Methyl bromide provided the best conditions for plant development as evaluated by plant height data during this time period (Figure 6.3-a). In March 1993, plants in limed plots grew better than those in nonamended plots, while plants growing in methyl bromide treated plots were 5.5 cm higher (21.7%) than those in limed plots ($P \leq 0.05$). This positive response in limed plots was obtained after four applications of pulverized dolomitic limestone, raising soil

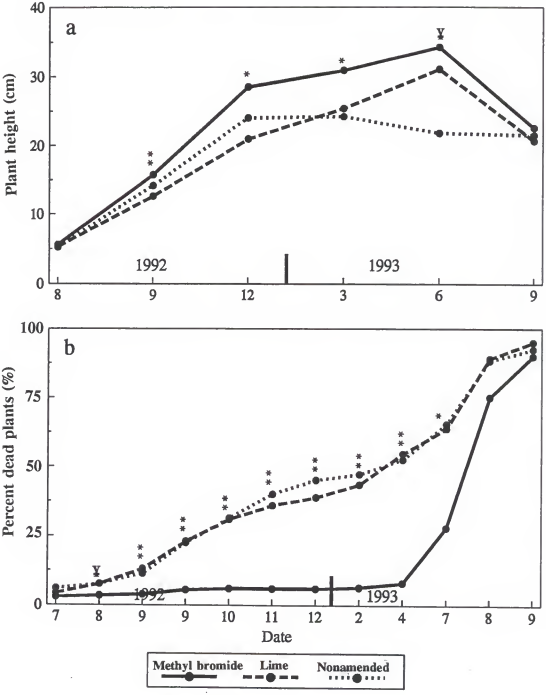
Table 6.1. Analysis of variance summary of the plant height data collected over time in the disease control study with sage[†].

| Source[§] | 1992 | | | 1993 | | |
|---|---|---|---|---|---|---|
| | 8/20 | 9/17 | 12/8 | 3/19 | 6/9 | 9/15 |
| | -------- level of significance[‡] ---------- | | | | | |
| Replication | n s | * * | * | * | n s | n s |
| Amendment (a) | n s | * * | * | * | ¥ | n s |
| Mulch (b) | n s | n s | n s | * * | * | n s |
| Interaction (axb) | n s | n s | n s | n s | n s | n s |
| CV (%) | 7.8 | 12.7 | 17.9 | 16.7 | 23.7 | 73.9 |

[†] ANOVA of a randomized complete block design arranged in split-plot with four replications on each date.

[‡] n s = F value non significant; ** = F value significant at $P \leq 0.01$; * = F value significant at $P \leq 0.05$; ¥ = F value significant at $P \leq 0.10$.

[§] Soil amendment treatments are methyl bromide, lime and nonamended; mulch treatments are organic mulch, synthetic mulch and nonmulch.



* * = F value significant at $P \leq 0.01$; * = F value significant at $P \leq 0.05$; and ¥ = F value significant at $P \leq 0.10$. Dates without a symbol showed no significant difference.

Figure 6.3. Effect of soil amendment on plant height (a) and number of dead plants (b) of sage.

pH to 6.6. By September 1993, the last date of data collection, average plant height had been severely reduced in all soil amendment treatments, as all plants deteriorated.

Plant mortality was affected by soil amendment treatments from August 1992 to July 1993 (Table 6.2). Methyl bromide provided the best conditions for survival at 9 of the 12 evaluation dates (Figure 6.3-b). During the same period, limed and nonamended plots seem to be influenced by seasonal changes. Plant losses were slightly less in limed plots than in nonamended plots during the fall and winter of 1992 (Figure 6.3-b).

Percent dead plants in methyl bromide plots increased 350% from April to July (8 to 28%) of 1993 (Figure 6.3). However, methyl bromide continued to favor survival, when compared to limed and nonamended plots ($P \leq 0.05$). By September 1993, all soil amendment treatments showed similar response with more than 90% dead plants. Thus, the fumigation effect of methyl bromide was lost about 1.5 year after application.

Mulch treatments affected plant height 11 months after the experiment began (Table 6.1; Figure 6.4). Organic mulch favored plant development over that in synthetic mulch and nonmulch treatments in March ($P \leq 0.01$) and June ($P \leq 0.05$) 1993 (Figure 6.4-a). No difference in plant height was detected between synthetic mulch and nonmulch treatments throughout

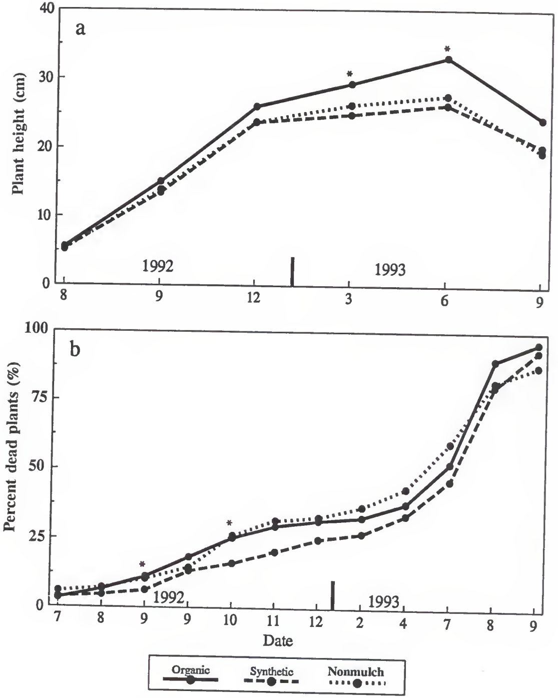
Table 6.2. Analysis of variance summary of percent dead plant data collected over time in the disease control study with sage.[†]

| Source[§] | 1992 | | | | | | | 1993 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 7/20 | 8/25 | 9/17 | 9/29 | 10/27 | 11/19 | 12/8 | 2/5 | 4/28 | 7/10 | 8/29 | 9/30 |
| | level of significance[‡] | | | | | | | | | | | |
| Replication | * * | * * | * * | n s | n s | n s | n s | n s | n s | n s | ¥ | ¥ |
| Amendment (a) | n s | ¥ | * * | * * | * * | * * | * * | * * | * * | * | n s | n s |
| Mulch (b) | n s | n s | * | n s | * | n s | n s | n s | n s | n s | n s | n s |
| Interact. (axb) | n s | * | ¥ | n s | n s | n s | n s | n s | n s | n s | n s | * |
| CV (%) | 72.4 | 71.3 | 55.8 | 54.5 | 39.2 | 50.2 | 54.4 | 53.6 | 43.7 | 27.5 | 14.6 | 9.7 |

† ANOVA of a randomized complete block design arranged in split-plots with four replications on each date.

‡ n s = F value non significant; ** = F value significant at *P*≤0.01; * = F value significant at *P*≤0.05; ¥ = F value significant at *P*≤0.10.

§ Soil amendment treatments are methyl bromide, lime and nonamended; mulch treatments are organic mulch, synthetic mulch and nonmulch.



* * = F value significant at $P \leq 0.01$; * = F value significant at $P \leq 0.05$; and ¥ = F value significant at $P \leq 0.10$. Dates without a symbol showed no significant differences.

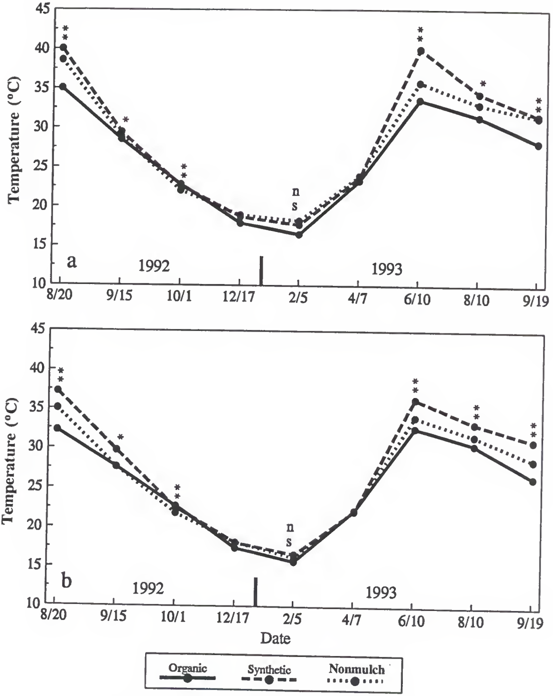
Figure 6.4. Effect of mulch treatments on plant height (a) and number of dead plants (b) of sage.

the experiment. By harvest in September 1993, the differences between mulch treatments were reduced. There was no interaction between soil amendment and mulch treatments for any date during the study, as expressed by plant height.

Mulch treatments affected plant mortality at the beginning of the experiment, in September and October 1992 (Table 6.2). In October 1992, synthetic mulch improved plant survival ($P \leq 0.05$) (Figure 6.4-b).

Mulch treatments affected soil temperature at 5-cm and 10-cm depth on most dates (Figure 6.5-a and 6.5-b). Independent of soil depth, mulch effect on temperature was more evident during the summer months (Appendix F-5 to F-8). Organic mulch maintained the coolest temperature for most dates, while soil under synthetic mulch remained the warmest during the summer; nonmulch plots were warmer during the fall and winter.

*Fusarium oxysporum* root rating data were analyzed because of a high level of root decay at harvest. The population density of *F. oxysporum* in the soil presented a different scenario. There were no effects of soil amendment treatments on the number of ppg (Appendices F-9 and F-10). However, the lower population densities associated with soil amendment treatments appeared to be under limed conditions for all mulch treatments.



n s = F value non significant; ** = F value significant at *P*≤0.01; and * = F value significant at *P*≤0.05. Dates without a symbol did not have ANOVA.

Figure 6.5. Effect of mulch treatments on soil temperature at 5-cm depth (a), and at 10-cm depth (b) in the disease control study with sage.

Mulch treatments affected the population density of *F. oxysporum* ($P \leq 0.10$), with synthetic mulch having the lowest mean (7,311 ppg), followed by organic mulch (11,627 ppg) and nonmulch (14,600 ppg) (Tables 6.3 and 6.4). Although, no interaction was detected, lime plus synthetic mulch had the lowest *F. oxysporum* population density, with 5,417 ppg for lime-mulch combinations. Engelhard (1989) found that rising soil pH to 7.0 or higher suppressed Fusarium wilt in chrysanthemum.

Sage roots had significant gall formation, which corroborated the findings of Moreno et al. (1992). The ANOVA showed differences among treatments for *M. incognita* gall rating ($P \leq 0.01$) (Tables 6.5 and 6.6). Methyl bromide and lime treatments combined with organic mulch produced the lowest gall index of 2.0. Since all plants in the nonamended plus nonmulch treatment died before harvest, it was not rated. However, the surviving nonamended treatments had high mean ratings (mean gall rating of 3.7). The highest average rating occurred in the nonmulched plots treated with methyl bromide (mean gall rating of 4.3) (Table 6.5).

Contrasts between soil amendment treatments showed that a greater gall rating occurred in the methyl bromide treatment than in the limed and nonamended treatments ($P \leq 0.05$) (Table 6.7), but that gall rating in limed plots

Table 6.3. *Fusarium oxysporum* population density in soil at harvest.

| Mulch treatment | Soil amendment | | | Average |
|---|---|---|---|---|
| | Methyl bromide | Lime | Nonamended | |
| | ppg† | | | |
| Organic | 16,600‡ | 7,632 | 10,650 | 11,627 |
| Synthetic | 8,783 | 5,417 | 7,733 | 7,311 |
| Nonmulch | 10,883 | 9,588 | 23,333 | 14,600 |
| Average | 12,089 | 7,544 | 13,907 | |

† The number of propagules per gram was determined by diluting 1 g of soil in 300 ml of autoclaved, deionized $H_2O$.

‡ Two replications of the field disease control study were sampled. Nine plates of Komada's medium were inoculated for each field treatment and replication for a total of 18 plates per treatment. The average for the two field replications is included in the table.

Table 6.4. Analysis of variance summary of the effect of *Fusarium oxysporum* population density in soil at harvest.†

| Source§ | Probability | Significance |
|---|---|---|
| Amendment (a) | 0.136 | n s‡ |
| Mulch (b) | 0.099 | ¥ |
| Interaction (axb) | 0.223 | n s |
| CV (%) | 109.4 | |

† ANOVA for a randomized complete block design with nine replications.

‡ n s = F value non significant; ¥ = F value significant at $P \leq 0.10$.

§ Soil amendment treatments are methyl bromide, lime and nonamended; mulch treatments are organic mulch, synthetic mulch and nonmulch.

Table 6.5. Gall rating of sage roots infested with *Meloidogyne incognita* at harvest.

| Mulch treatment | Soil amendment | | | Average |
|---|---|---|---|---|
| | Methyl bromide | Lime | Nonamended | |
| | ---------- Gall rating† ------------- | | | |
| Organic | 2.0‡ | 2.0 | 3.0 | 2.3 |
| Synthetic | 3.3 | 3.0 | 3.3 | 3.2 |
| Nonmulch | 4.3 | 3.0 | - | 3.7 |
| Average | 3.2 | 2.7 | 3.2 | |

† Gall rating: 0 = 0, 1 = 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100, 5 = >100 galls per plant.

‡ Three replications from the disease control study were harvested. The combination of nonamended plus nonmulch (control) was not evaluated because all plants had died before harvest.

Table 6.6. Analysis of variance summary of root gall rating response across all treatments for sage at harvest.[†]

| Source | Probability | Significance |
|---|---|---|
| Replications | | n s[‡] |
| Disease control treatment | .002 | * * |
| CV (%) | 17.82 | |

† ANOVA for a complete randomized design with three replications.

‡ n s = F value non significant; ** = F value significant at $P \leq 0.01$.

was not different than that in nonamended plots. This implies that, after about 14 months, methyl bromide effects had been lost and there was possibly a resurgence of the *M. incognita* population.

Contrasts among mulch treatments showed that gall rating in organic mulch was lower than that in synthetic mulch and nonmulch treatments; thus, root galling was reduced with the application of organic mulch ($P \leq 0.01$) (Table 6.7). Root galling in synthetic mulch was not

Table 6.7. Single degree of freedom orthogonal contrasts for summary of gall rating for sage at harvest.

| Contrast† | Probability | |
|---|---|---|
| | Value | Significance |
| Soil amendment treatments: | | |
| Methyl bromide vs. Lime | 0.044 | *‡ |
| Methyl bromide vs. Nonamended | 0.040 | * |
| Lime vs. Nonamended | 0.097 | n s |
| Mulch treatments: | | |
| Organic vs. Synthetic | 0.003 | * * |
| Organic vs. Nonmulch | 0.000 | * * |
| Synthetic vs. Nonmulch | 0.136 | n s |
| Combinations of treatments: | | |
| Methyl bromide + Organic vs. Lime + Organic | 0.000 | * * |
| Methyl bromide + Synthetic vs. Lime + Synthetic | 0.580 | n s |

---

† The single degree of freedom contrasts were done to determine significance of variance between treatments.

‡ n s = F value not significant; ** = F value significant at $P \leq 0.01$; * = F value significant at $P \leq 0.05$.

different from that in nonmulch treatments. The combination of methyl bromide plus organic mulch was different from the the combination of lime plus organic mulch ($P \leq 0.01$), but the combination of methyl bromide plus synthetic mulch was not different from lime plus synthetic mulch.

At harvest, the population density of *M. incognita* showed no response to soil amendment and mulch treatments (Tables 6.8 and 6.9). However, there was an interaction effect between mulch and soil amendment ($P \leq 0.10$). The combination of lime plus synthetic mulch had the lowest population density of the experiment, with 26 J2 100 ml$^{-1}$ of soil. The combination of methyl bromide plus synthetic mulch had the highest population density, with 271 J2 100 ml$^{-1}$ of soil, followed by methyl bromide plus nonmulch, with 218 J2 100 ml$^{-1}$ of soil. These populations may have resulted from a resurgence of *M. incognita* after the effects of fumigation wore off.

McSorley and Gallaher (1994), at a nearby site, found that liming had no effect on *M. incognita* population density. However, there is the possibility that frequent applications of dolomitic limestone at the rate of 16.5 metric tons ha$^{-1}$ year$^{-1}$ could have suppressed nematode population. When the synthetic mulch was removed from plots treated with lime, a concentration of dolomitic limestone was observed in the top 5 cm of soil. Also, a concentration and proliferation of roots was observed within this soil

Table 6.8. Population density of *Meloidogyne incognita* in soil of the disease control study with sage at harvest.

| Mulch treatment | Soil amendment | | | Average |
|---|---|---|---|---|
| | Methyl bromide | Lime | Nonamended | |
| | ------------ # J2 100 $ml^{-1}$ ------------ | | | |
| Organic | 85[‡] | 67 | 63 | 72 |
| Synthetic | 271 | 26 | 37 | 111 |
| Nonmulch | 218 | 50 | 72 | 113 |
| Average | 191 | 48 | 57 | |

[†] The number of juveniles (J2) was determined by centrifugal flotation using 100 ml of soil.

[‡] Only two replications from the field disease control study were sampled at harvest.

Table 6.9. Analysis of variance summary for soil population density of *Meloidogyne incognita* at harvest.[†]

| Source[§] | Probability | Significance |
|---|---|---|
| Amendment (a) | 0.228 | n s[‡] |
| Mulch (b) | 0.358 | n s |
| Interaction (axb) | 0.090 | ¥ |
| CV (%) | 52.52 | |

[†] ANOVA as a randomized complete block design arranged in split-plots with two replications.

[‡] n s = F value not significant; ¥ = F value significant at $P \leq 0.10$.

[§] Soil amendment treatments are methyl bromide, lime and nonamended; mulch treatments are organic mulch, synthetic mulch and nonmulch.

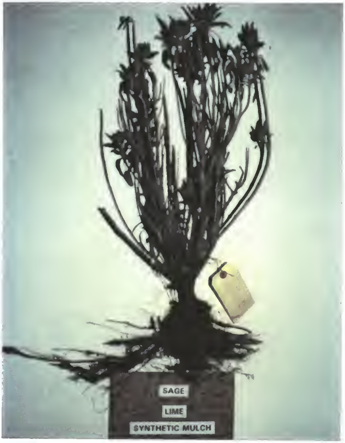


Figure 6.6. Sage plant extracted at harvest from a plot treated with lime plus synthetic mulch.

zone (Figure 6.7). Shoot and root dry weights showed no difference among treatments (Appendix F-9 to F-12).

In conclusion, the die-back disease of sage was severe in all disease control treatments by the end of the test. There were indications of improvement in plant development, plant survival, and reduced root infection by *M. incognita* under methyl bromide and organic mulch. Synthetic mulch appears to reduce *F. oxysporum* population density, especially in combination with lime. None of the treatments over time provided adequate protection, and 90% of the plants were dead by the end of the test.

Galls from *M. incognita* were found on field grown sage. These findings agree with results obtained from glasshouse pathogenicity studies and previous findings by Moreno et al. (1992). Given the environmental factors, the cultural practices, and genotypes of sage used in this research, it appears that commercial field production of sage is not practical for Florida. Based on the results of this study, future work on the field production of sage in Florida should focus on a search for plant nematode resistance, more extensive use of organic amendment soil treatment, and fungicides.

# CHAPTER VII
# DISEASE CONTROL STUDY WITH LAVENDER

## Introduction

Lavender (*Lavandula* spp.) is a perennial shrub native to the south coast of France. Duke and Hurst (1975), using the Holdridge (1966) life zones system, considered lavender to be adapted for growth from the Cool Temperate Moist Forest life zone (6-12ºC; 500-1,000 mm of rain yr$^{-1}$) to the Warm Temperate Moist Forest life zone (18-24ºC; 1,000-2,000 mm of rain yr$^{-1}$). The cultivar used in this research was identified by Tucker et al. (1993) as *Lavandula x hybrida* Balb. ex Ging. This hybrid is considered of commercial importance in the essential oil market.

De Baggio (1987) stated that lavender tolerates a range of soil pH from 5.8-8.3, but growth is best in alkaline soils. Robbins (1988) reported that lavender grows best and the yield is better with an application of 13 to 17 metric tons of dolomitic limestone ha$^{-1}$ year$^{-1}$. Engelhard and Wolz (1973) found that raising soil pH to 7.0 reduced the level of *Fusarium* wilt in Chrysanthemum (*Chrysanthemum* spp.). Bruehl (1987) warned that it is rarely practical to control soilborne diseases by modifying soil pH alone.

Tucker and Maciarello (1990b) considered that, under hot and humid conditions, lavender becomes susceptible to soilborne fungi. Angell (1991) observed a high incidence of a die-back disease of lavender at the Green Acres Agronomy Farm, Gainesville, FL. Lavender plants appeared stunted with root decay and black lesions extending up the main stem from the soil line. Espaillat et al. (1993) obtained 18 isolates of *Fusarium oxysporum* Link from plants grown in a glasshouse in infested soil collected from the site where Angell (1991) made his observations. Searches of the published literature by the senior author and by Simon et al. (1984) failed to find previous reports of disease caused by *F. oxysporum* on lavender.

Hasseb and Pandley (1987) concluded that root-knot nematodes (*Meloidogyne* spp.) are an important constraint in the production of several medicinal and aromatic plants. Lisetskaya (1973) observed that *M. hapla* Chitwood caused moderate to severe growth reduction of lavender. Moreno et al. (1992) found sage plants to have high gall ratings and egg mass indices resulting from inoculation with *M. incognita* race 1.

Balbaa (1983) and Latin (1986) found a scarcity of specific information on herb production techniques and disease control, including few registered fungicides. An index of scientific publications on herbs, aromatic and medicinal plants was compiled by Simon et al. (1984). In the section of plant protection and disease control, only 99 (41%) of 239 publications dealt with general disease control measures. Three publications evaluated pests or diseases of lavender. Bassino et al. (1975) reported on control of leaf midge; Maia et al. (1973) discussed the general plant health of Abrial lavandin; and by Zhukova (1974) evaluated control of septoriosis disease.

Romanenko and Zhokora (1975) investigated the heritability of resistance of lavender hybrids to leaf spot, and Zhukova (1975) evaluated the resistance of lavender cultivars to septoriosis. An integrated disease control system for lavender was evaluated by Tsalbukov (1973). All of the above citations came from Russian and French journals. On a world scale there is a lack of scientific literature on the use of cultural practices for herbs. This is especially important when considering that Morris and Cracker (1990), as well as many herb producers, associate herb production in general to the organic farming philosophy.

Trivedi and Barker (1986) suggested the development of integrated disease management strategies, which include cultural practices to replace soil fumigation. Kharbanda and Tewari (1996) considered that most cultural practices aim at reducing the amount of primary inoculum in the soil. Palti (1981) estimated that through suitable adjustments and combinations of cultural practices, it is possible to modify the environment so that conditions become unfavorable for one or more pathogens and subsequent disease development.

Rich (1986) considered the suppression of soilborne fungi and nematodes to be important benefits of mulching, which improves plant development and yield as a result of nutrient release and changes in soil microclimate. Aust et al. (1980) and Huber and Watson (1970) determined that organic mulches raise the "biological buffering capacity" by enriching the soil with microflora competitive or antagonistic to pathogens.

To develop a better understanding of the die-back disease of field grown lavender, and to provide herb growers in North-Central Florida with information on alternative disease control methods, the following objectives were established: 1) assess the die-back disease of lavender over time, 2) evaluate the effect of soil management practices on growth and survival of lavender, and 3) evaluate the effect of manipulation of soil microclimate in the control of *Fusarium oxysporum* Link and *Meloidogyne incognita* Kofoid and White race 1.

## Materials and Methods

A field disease control study was conducted from April 1992 to September 1994 at the Green Acres Agronomy Farm, 22 km west of Gainesville, Florida. The soil at the experimental site was classified as Arredondo fine sand (loamy siliceous, hyperthermic family of Arenic and Grossarenic Paleudults). Angell (1991) reported a high incidence of a die-back disease in sage grown at the site during previous research (Figure 7.1).

The experimental area was kept under annual ryegrass (*Lolium multiflorum* Lam.) during the winter and fallowed during the summer for 2 years presiding establishment of the disease control study. In February 1992, the study area was plowed, followed by a disc harrow.

## Experimental Design

The experiment was established in April 1992 using a randomized, complete block design arranged in split-plots with five replications. Main plots consisted of three soil amendment treatments: methyl bromide, lime, and nonamended. Subplots were three mulch treatments: organic mulch, synthetic mulch, and nonmulch (Figure 6.2). The subplots were 2.75 $m^{-2}$, containing 20 plants arranged in four rows with five plants per row. Rows were spaced 0.45 m apart with 0.30 m between plants in a row.

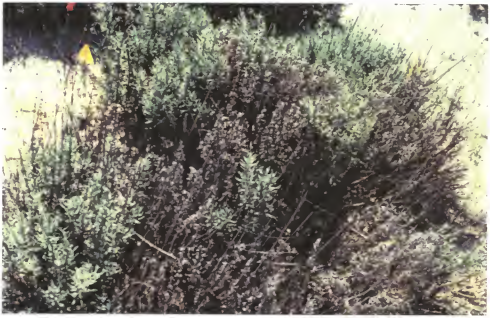


Figure 7.1. Diseased lavender plants grown at the Green Acres Agronomy farm, Gainesville, Florida.

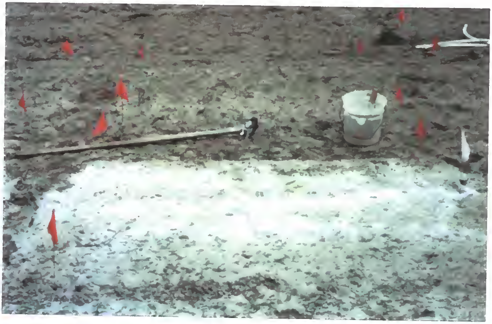


Figure 7.2. Lime application at establishment of the disease control study with lavender at the Green Acres Agronomy farm, Gainesville, Florida.

Methyl bromide (Brom-o-gas® = 98% methyl bromide, 2% chloropicrin) was applied at a rate of 1.1 kg in 9.18 $m^2$. Prior to methyl bromide application, the area was irrigated with 50 mm of water. Methyl bromide was applied in a sealed chamber, constructed with polyethylene sheets placed over the plot area. After 2 days the polyethylene sheets were removed, allowing the soil to aerate for 15 days prior to transplant.

Lime treatment plots were maintained at pH ± 7.2. Adjustment of soil pH was based on calculations using the method developed by Adams and Evans (1992) and average soil pH. Pulverized dolomitic limestone was applied uniformly across the whole plot. Six applications of 5,570 kg $ha^{-1}$ (5.5 metric tons) each were made to raise and maintain soil pH close to 7.2 during the 2-year study (Appendix C-4). The first lime application was done 2 months before transplant (Figure 7.2). The need for subsequent lime applications was determined by soil pH monitoring. Nonamended plots were maintained with a bare soil surface.

Dry, shredded perennial peanut (*Arachis glabrata* Benth) hay, fumigated with methyl bromide at a rate of 1 kg fumigant to 4 $m^3$ served as the organic mulch. The first organic mulch application of 3,300 kg $ha^{-1}$ was made after sage transplant, forming a ground cover 3 cm thick. Four subsequent applications of 2,200 kg $ha^{-1}$ each (totalling 12.2 metric tons of hay mulch $ha^{-1}$ $year^{-1}$) were applied as needed on top of the previous decomposing hay mulch to maintain a constant organic mulch cover. During the second year, three applications of 2,200 kg ha$^{-1}$ each were made for a total of 6,600 kg (7.3 tons) ha$^{-1}$ year$^{-1}$.

The synthetic mulch treatment was "pro5 Weed Barrier"® (DeWitt company, Sikeston, Missouri), a black and woven polypropylene ground cover that stops weeds and allows water and nutrients to pass through. Because of the porous nature of Pro5, the use of overhead irrigation and over the top applications of lime, fertilizers and fungicides were possible.

Synthetic mulch material was cut into 1.9- X 2.1-m pieces to fit over the appropriate treatment plots. Holes (12-cm diameter) were melted in the Pro5 material with a propane torch to accommodate a transplant. The synthetic mulch was placed over the plot area and secured with lose soil along the perimeter edges of the mulch.

Nonmulched treatment plots were maintained free of weeds and other organic residues. The nonamended plus nonmulched treatment combination served as the control for analysis of response variables.

In June 1992, healthy appearing 1-month-old rooted cuttings of lavender were transplanted. Overhead sprinkler irrigation was provided as needed. Fertilizer was applied every 8 weeks in accordance with Angell's (1991) findings of 250 kg of nitrogen ha$^{-1}$ year$^{-1}$ for lavender grown at the same site. The total amount of fertilizer applied year$^{-1}$ was split in six applications, the first after transplant with subsequent applications every 2 months. Pure sand was mixed with the fertilizer, at a proportion of 2:1, to facilitate uniform distribution over the entire plot.

Plots were kept weed free by directed applications of Roundup (Glyphosate) as often as required at a dosage of 13 ml liter$^{-1}$ of tap water, plus 13 ml of non-ionic surfactant liter$^{-1}$ of tap water. Herbicide application was made using a 7.5-liter $CO_2$ powered sprayer. Roundup was applied eight times, six times during the first year and five times during the second year. Between herbicide applications weeds were removed by hand and a hoe.

## Data Collection

Following application of soil amendment treatments (August 1992), population density of *F. oxysporum* was determined for each plot by plating soil samples on Komada's (1975) specific medium for *F. oxysporum*. After 7 days of incubation at 27ºC with 12 hours of light and 12 hours of darkness, the numbers of *F. oxysporum* ppg of soil were counted (Appendix B-2).

Parameters recorded periodically during the experiment included plant height (mean of four plants per plot), percent dead plants, and soil temperature at 5- and 10-cm depths. Parameters evaluated during the destructive sampling in April 1994 included stem diameter, shoot dry weight, root dry weight, *M. incognita* gall rating, *F. oxysporum* root rating and percent root infection by *F. oxysporum*.

Plant height was measured from the soil-line at the base of the stem to the tip of the highest leaf using five replications. Soil temperature was measured using four replications in the first year and three replications in the second year with a digital electronic recording device with a soil probe placed at 5- and 10-cm depths.

Lavender spikes were harvested in four replications when 75% of the spikelets were opened following established commercial practices (Lammerink et al., 1989, and Morton, 1976). Under the environmental conditions at the Green Acres Agronomy Farm and agronomic practices provided for this study, optimum flowering stages for harvests occurred on 4 June 1993, and 7 June 1994. To harvest the spikes, a template frame was made, 120 cm long and 50 cm wide, for a harvesting area of 0.6 $m^2$. Spike length was obtained by measuring 20 of the largest top spikes per plot, and calculating the mean spike length.

On April 1994, a destructive sample was taken by harvesting one replication. Whole plants were extracted from soil, washed, and evaluated for plant infection by *F. oxysporum* and *M. incognita*. Agronomic parameters evaluated in the destructive sampling included stem diameter, shoot dry weight and root dry weight.

Stem diameter was measured at the soil-line with a caliper. Shoots and roots were separated and dried at 70ºC for 4 days in an forced-air oven, and weighed.

Roots were rated for *M. incognita* gall formation based on a 0-5 rating scale, where 0 = 0 galls, 1 = 1-2 galls, 2 = 3-10 galls, 3 = 11-30 galls, 4 = 31-100 galls, and 5 = more than 100 galls per plant root system (Taylor and Sasser, 1978) (Appendix C-3). Because galls caused by *M. Incognita* did not occur on lavender roots, it was not necessary to determine the population density of the nematode in the soil. The population density of *F. oxysporum* in the soil was not determined either; rather roots were rated for necrosis and decay caused by *F. oxysporum* on a scale of 1-6, where 1 = white, healthy looking roots without adventitious roots; 2 = white, healthy looking roots with adventitious roots; 3 = white, healthy looking roots with adventitious roots and yellowish plant foliage; 4 = some brown roots with adventitious roots and yellowish and dwarf plants; 5 = many brown roots without adventitious roots, and dwarf and wilted plants; and 6 = mostly brown roots without adventitious roots, and dying plants (Appendix C-2). The percent of *F. oxysporum* root infection was determined by plating a subsample of seven 1.3-cm-long root pieces in Komada's

(1975) medium, incubating at 27°C with light and dark cycled every 12 hours, and counting the number of infected root pieces after 7 days.

## Statistical Analysis

Data were analyzed using analysis of variance of a randomized complete block design arranged in split-plots. *Meloidogyne incognita* gall rating, shoot dry weight, root dry weight, and stem diameter were not analyzed, because only one replication from the disease control study was sampled in April 1994. All data were analyzed using the MSTAT-C (1992) statistical program.

## Results and Discussion

Soil population density of *F. oxysporum* 2 months after application of methyl bromide in treatment plots was 280 ppg soil. *Fusarium oxysporum* population densities found present in lime treated plots (1,480 ppg) and nonamended plots (1,760 ppg) gave evidence of the effectiveness of methyl bromide in reducing the pathogen (Appendix C-4).

Plant height was not affected by soil amendment treatments at any time during the 2-year study (Table 7.1). Even though methyl bromide reduced plant mortality, its effect on plant height was not different from that in lime and nonamended treatments (Figure 7.3-a; Appendix G-1).

Table 7.1. Analysis of variance summary of plant height data collected over time in the disease control study with lavender.

| Source§ | First Year | | | | | | | Second Year | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1992 | | | 1993 | | | | 1994 | | |
| | 8/20 | 9/17 | 12/8 | 3/19 | 6/9 | 9/15 | 12/20 | 2/15 | 5/10 | 7/15 |
| | level of significance† | | | | | | | | | |
| Replication | * *‡ | * * | * * | n s | * * | ¥ | n s | ¥ | * | * * |
| Amendment (a) | n s | n s | n s | n s | n s | n s | n s | n s | n s | n s |
| Mulch (b) | * * | * * | * * | * * | * * | * * | * * | * | n s | n s |
| Interaction (axb) | n s | n s | n s | ¥ | * | * | * * | * | * | * |
| CV (%) | 17.70 | 14.60 | 15.45 | 11.29 | 11.04 | 11.70 | 10.55 | 10.32 | 10.60 | 10.89 |

† ANOVA of a randomized complete block design arranged in split-plots with five replications.

‡ n s = F value non significant; ** = F value significant at $P \leq 0.01$; * = F value significant at $P \leq 0.05$; ¥ = F value significant at $P \leq 0.10$

§ Soil amendment treatments are methyl bromide, lime and nonamended; mulch treatments are organic mulch, synthetic mulch and nonmulch.

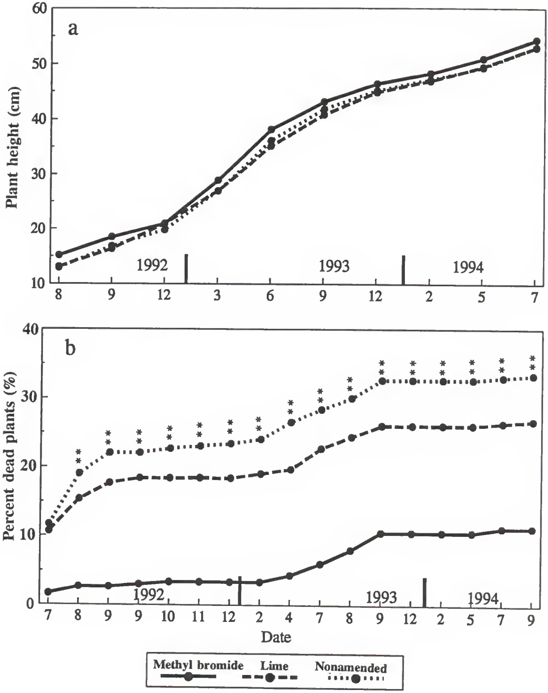
Table 7.2. Analysis of variance summary of the percent dead plant data collected in the disease control study with lavender.

| Source§ | First Year | | | | | | | | | | | | | Second Year | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1992 | | | | | | | 1993 | | | | | | 1994 | | | |
| | 7/20 | 8/25 | 9/17 | 9/29 | 10/27 | 11/19 | 12/8 | 2/5 | 4/28 | 7/10 | 8/29 | 9/30 | 12/20 | 2/15 | 5/10 | 7/15 | 9/10 |
| | level of significance† | | | | | | | | | | | | | | | | |
| Replication | n s‡ | n s | ¥ | * | * | * | * | * | n s | n s | n s | n s | n s | n s | n s | n s | n s |
| Amendment (a) | ¥ | * * | * * | * * | * * | * * | * * | * * | * * | * * | * | * | * * | * | * | * | * |
| Mulch (b) | n s | n s | n s | n s | n s | n s | n s | n s | n s | n s | n s | n s | n s | n s | n s | n s | n s |
| Interaction (axb) | n s | n s | n s | n s | n s | n s | n s | n s | n s | n s | n s | n s | n s | n s | n s | n s | n s |
| CV (%) | 93.8 | 67.2 | 61.9 | 62.9 | 61.6 | 61.5 | 62.1 | 63.0 | 63.5 | 66.6 | 67.2 | 62.3 | 62.3 | 62.3 | 62.3 | 62.5 | 62.4 |

† ANOVA of a randomized complete block design arranged in split-plots with five replications.

‡ n s = F value non significant; ** = F value significant at $P \leq 0.01$; F value significant at $P \leq 0.05$; and ¥ = F value significant at $P \leq 0.10$.

§ Soil amendment treatments are methyl bromide, lime and nonamended; mulch treatments are organic mulch, synthetic mulch and nonmulch.

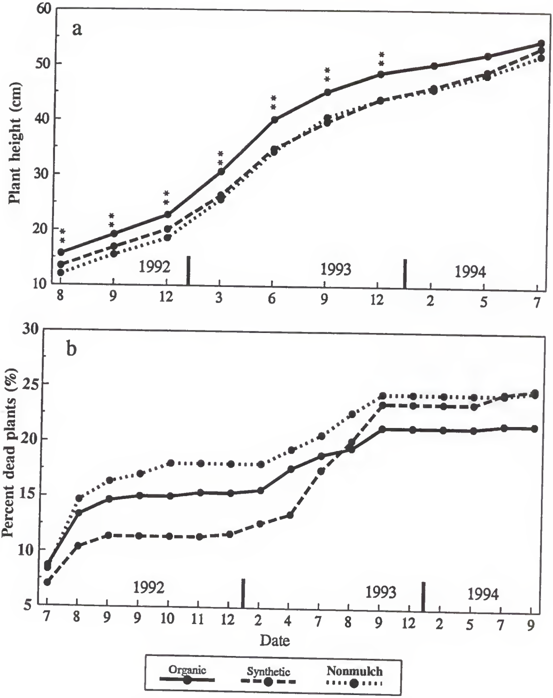


* * = F value significant at $P \leq 0.01$. No symbol at a date means there was not a significant difference.

Figure 7.3. Effect of soil amendment on plant height (a) and percent dead plants (b) with lavender.

The 2-year study clearly demonstrated that plant mortality was a function of the soil amendment treatments (Table 7.2). There were highly significant differences ($P \leq 0.01$) in 16 of the 17 dates evaluated (Figure 7.3-b). From September 1992 to February 1993, the plant mortality in methyl bromide treated plots remained below 4.0 percent dead plants ($P \leq 0.01$) (Figure 7.3-b). During the same period, the response in limed plots and nonamended plots increased to 19 and 24 percent dead plants, respectively. During the second year, plant survival continued to be greater in plots treated with methyl bromide (Table 7.2). In September 1994, at the end of the 2-year study, methyl bromide plots had 11 percent dead plants, while limed and nonamended plots had 27 and 34 percent dead plants, respectively ($P \leq 0.01$) (Appendix G-5). The lime treatment appeared to provide better conditions than nonamended soil for survival (Figure 7.3-b). Engelhard (1989) concluded that liming to pH 7.0 improved survival by reducing Fusarium wilt on many crops in South Florida.

The effect of mulch treatments on plant height was significant during the first 16 months of the 2-year study (Table 7.1). From August to December 1992, organic mulch appeared to favor plant development over that in synthetic mulch and nonmulch treatments (Figure 7.4-a; Appendix G-2). No differences between mulch treatments were detected after



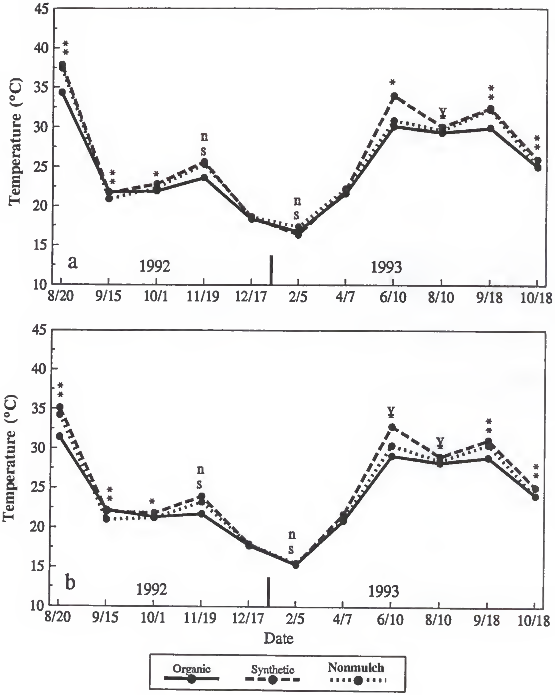
* * = F value significant at $P \leq 0.01$; and * = F value significant at $P \leq 0.05$. No symbol at a date means there was not a significant difference.

Figure 7.4. Effect of mulch treatments on plant height (a) and percent dead plants (b) with lavender.

February 1994. It was observed that plant canopy closure occurred about the same time that the mulch effect diminished, minimizing any mulch effect. Interactions between soil amendment and mulch treatments were detected from March 1993 until the end of the study in July 1994 (Table 7.1). The interaction effect appeared to be in response to plant height changes in synthetic and nonmulch treatments. Plant mortality was not affected by mulch treatments (Table 7.2; Figure 7.4-b).

Soil temperatures were affected by mulch treatments at 5- and 10-cm depths. The effect of mulches on soil temperature was season dependant (Figure 7.5-a and b). Independent of soil depth, mulch effect was more apparent during the summer months (Appendix G-7 to G-10). Organic mulch generally maintained the coolest temperature throughout the 14 months of data collection, while synthetic mulch usually resulted in the highest temperatures during the summer.

Spike weight was affected by mulch treatments in the first harvest ($P \leq 0.10$) (Table 7.3). The organic mulch treatment had a mean yield of 656.5 g $m^{-2}$, followed by nonmulch with 523.2 g $m^{-2}$ and synthetic mulch with 472.3 g $m^{-2}$ (Appendix G-11). The combination of lime plus organic mulch yielded 816.5 g $m^{-2}$, which was 32% higher than the next highest treatment, organic mulch (619.0 g $m^{-2}$). Spike length



n s = F value non significant; ** = F value significant at *P*≤0.01; * = F value significant at *P*≤0.05; and ¥ = F value significant at *P*≤0.01. Dates without a symbol did not have ANOVA.

Figure 7.5. Effect of mulch treatments on soil temperature at depths of 5-cm (a) and 10-cm (b) in the disease control study with lavender.

Table 7.3. Analysis of variance summary of spike weight and spike length for the first and second lavender harvest.[†]

| Source[§] | First Harvest | | Second Harvest | |
|---|---|---|---|---|
| | Weight | Length | Weight | Length |
| | --------- significance level[‡] | | ----------- | |
| Replications | n s | n s | n s | ¥ |
| Amendment (a) | n s | n s | n s | n s |
| Mulch (b) | ¥ | n s | n s | * |
| Interaction (axb) | n s | n s | n s | n s |
| CV (%) | 36.34 | 10.57 | 27.90 | 7.63 |

[†] ANOVA of a randomized complete block design arranged in split-plots with four replications in the first harvest and five replications in the second harvest.

[‡] n s = F value non significant; * = F value significant at $P \leq 0.05$; ¥ = F value significant at $P \leq 0.10$.

[§] Soil amendment treatments are methyl bromide, lime and nonamended; mulch treatments are organic mulch, synthetic mulch and nonmulch.

was not affected by soil amendment or mulch treatments during the first harvest (Table 7.3).

No effect of soil amendment was detected with either spike weight or spike length during the second harvest (Table 7.3). Mulch treatments affected spike length in the second harvest ($P \leq 0.05$) (Table 7.3). Plants in the nonmulch treatment had the longest spikes (35.5 cm), followed by those in synthetic mulch (34.5 cm) and organic mulch (32.8 cm) (Appendix G-14).

Rating of *F. oxysporum* root infection responded to soil amendment and mulch treatments ($P \leq 0.01$) (Table 7.4 and 7.5). Interaction between treatments was detected ($P \leq 0.01$). Among soil amendment treatments, lime had the lowest root infection with an average of 40.8%, followed by methyl bromide and nonamended with 48.8% and 65.6%, respectively (Table 7.4). Among mulch treatments, synthetic mulch resulted in the lowest root infection (31.1%), followed by organic mulch and nonmulch with 60.0% and 63.0%.

Methyl bromide plus synthetic mulch (9.1%) resulted in the lowest root infection. It appears that synthetic mulch played an important role, possibly by reducing soil contamination by *F. oxysporum* after fumigation. Lime plus synthetic mulch was the next best treatment with a root infection rating of 29.5%. The other treatment combinations tested yielded root infection greater than 45%, with methyl bromide plus nonmulch the highest at 82.3%.

No gall formation was observed on lavender roots sampled in April 1994 (Appendix G-15). Shoots obtained from the same plants sampled for gall rating, demonstrated the tendency of lime amendment to increase shoot weight (799 g plant$^{-1}$). Among mulch treatments, organic mulch produced the highest mean shoot weight with 741 g plant$^{-1}$. The treatment combinations of organic mulch with lime and organic mulch plus nonamended produced the heaviest shoots (890 and 870 g plant$^{-1}$) (Appendix G-16). The treatment combinations, organic mulch with lime and organic mulch with nonamended yielded the heaviest roots (299 and 203 g plant$^{-1}$). The data provide some indication that organic mulch across soil

Table 7.4. *Fusarium oxysporum* infection of lavender roots in April, 1994.

| Mulch treatment | Soil amendment | | | Average |
|---|---|---|---|---|
| | Methyl bromide | Lime | Nonamended | |
| | %† | | | |
| Organic | 54.9‡ | 47.1 | 80.9 | 60.9 |
| Synthetic | 9.1 | 29.5 | 54.7 | 31.1 |
| Nonmulch | 82.3 | 45.7 | 61.2 | 63.1 |
| Average | 48.8 | 40.8 | 65.6 | |

† The percent of infection was determined by plating between 12 and 19 root pieces on Komada's medium, counting the number infected and calculating the percentage of infectation.

‡ One replication of the field disease control study was sampled. Six plates of Komada's medium were inoculated for each treatment. The average for the six replications was obtained and they are the values included in the table.

Table 7.5. Analysis of variance summary of the effects of *Fusarium oxysporum* infectation of lavender roots at harvest (April, 1994).†

| Source | Probability | Significance |
|---|---|---|
| Amendment (a) | 0.001 | * * |
| Mulch (b) | 0.000 | * * |
| Interaction (axb) | 0.000 | * * |
| CV (%) | 18.30 | |

† ANOVA as a randomized complete block design arranged in split plots with six replications.

* * = F value significant at $P \leq 0.01$.

amendment treatments produced the heaviest root systems. Perennial peanut mulch combined with lime appeared to provide favorable conditions for root development.

Stem diameter measured from plants sampled in April 1994 provided some indication that lime treatment produced the greatest stem diameter (Appendix G-18) (56.13 mm diameter), followed by that in nonamended and methyl bromide treatments (41.83 mm and 37.50 mm). Among mulch treatments, the largest diameters were produced by plants in organic mulch (54.53 mm), followed by those in synthetic mulch and nonmulch treatment (41.27 mm and 39.67 mm). The data provide some indication that the treatment combinations lime plus synthetic mulch and lime plus organic mulch produced the largest diameter stems (64.10 mm and 63.30 mm).

In conclusion, methyl bromide enhanced plant survival during the 2-year study. Soil amendment treatments had no affect on lavender plant height. The combination of methyl bromide with synthetic mulch reduced root infection by *F. oxysporum*. Organic mulch increased lavender plant height up to the point of canopy closure, but had no apparent effect on plant survival, and organic mulch in combination with lime appeared to improve spike weight during the first year. Improvement in plant development and yield of lavender treated with organic mulch could have resulted from the contribution of approximately 200 kg of nitrogen $ha^{-1}$ $year^{-1}$

from the mulch in addition to generally lower soil temperatures.

Study findings point to *F. oxysporum* as the pathogen infecting lavender roots. Lavender is resistant to *M. incognita*. The chemotype used in this research contains 7% of the terpenoid linalool, considered by Hasseb et al. (1987) and Chatterjee et al. (1982) to have nematicidal properties. This study provide evidence that the die-back disease of lavender can be reduced with the use of an integrated control system which includes soil fumigation with methyl bromide, liming, and synthetic and organic mulch.

# CHAPTER VIII

# GENERAL DISCUSSION AND SUMMARY

## Pathogenicity Studies

*Fusarium oxysporum* Link was isolated from diseased plants of sage and lavender. A preliminary pathogenicity test with isolate 5 of *F. oxysporum* reduced sage plant height, shoot fresh weight and root fresh weight at all inoculum levels (300, 3,000 and 15,900 propagules per gram of soil). Results from contrasted means for percent dead plants revealed a 30% increase in mortality between the control (0 ppg) and 300 ppg of soil. The highest mortality, 70%, occurred at 15,900 ppg of soil. A preliminary pathogenicity test with lavender revealed no effect of isolate 2 of *F. oxysporum* on plant mortality, shoot and root fresh weight, and plant height. However, root disease rating was increased by *F. oxysporum* inoculum levels. Contrast of 0 and 6,900 ppg of soil showed *F. oxysporum* reduced plant height 60 days after establishment. Based on results of the preliminary pathogenicity test, *F. oxysporum* caused disease of sage and lavender in varying degrees, and lavender appears to be more tolerant than sage to the isolates of *F. oxysporum* tested.

*Meloidogyne incognita* race 1 was isolated from galled roots on diseased field-grown sage. The identification of *M. incognita* race 1 in sage based on a differential host test corroborated findings by Moreno et al. (1992). However, in the present study no galls were observed in field grown lavender in either the combined pathogenicity tests or the field disease control study.

### Combined Pathogenicity Tests with Sage and Lavender

Due to little or no disease expression and the lack of consistency of data between test 1 and 2, it cannot be concluded that die-back of sage is caused by *F. oxysporum*, *M. incognita* race 1, or an interaction of the two organisms. In test 2, an interaction effect between *F. oxysporum* and *M. incognita* race 1 was demonstrated only for plant height. Plant mortality in test 1 was reduced at final harvest by increasing levels of *M. incognita*; however, this was not confirmed in test 2. At 123 days, plants grown in soil infested with *F. oxysporum* exhibited etiolation with increased plant height and internode length in both tests. At 247 days after infestation, the early stimulatory effect of *F. oxysporum* on plant height gave way to a plant height reduction effect of *M. incognita* at the end of the tests.

Due to little or no disease expression and the lack of consistency of data between test 1 and 2, it cannot be concluded that die-back of lavender is caused by *F.*

*oxysporum*, *M. incognita* race 1, or an intearction of the two organisms. Although not validated in the second test, *M. incognita* alone, reduced plant height, canopy width, shoot and root dry weight in test 1. However, there were few galls on roots of plants in the glasshouse or in field grown lavender plants. In test 2, *F. oxysporum* reduced plant mortality and stimulated plant etiolation and flowering.

Response differences between test 1 and 2 in the combined pathogenicity tests for both sage and lavender can perhaps be traced to the difference in environmental conditions of the two glasshouses used. Maximum and minimum temperatures between the glasshouses were similar; however, test 1 was conducted under higher relative humidity inherent under the evaporative-cooled system, while test 2 was conducted under reduced relative humidity characteristic of a refrigerated-air-cooled environment. Also, the original soil used in the preliminary test was stored for one year before being used in the combined pathogenicity study, and there is the possibility that *F. oxysporum* effectiveness was lost during storage, which could explain the lack of mortality (less than 7%), root weight loss, or validated results between the combined pathogenicity tests.

## Disease Control Studies

Methyl bromide reduced limitations on plant development and survival of sage during the first 10 months of the experiment, but die-back in all treatments was severe by the end of the test.

Sage grown in soil treated with lime paralleled plant height increases observed in methyl bromide treated plots for the first 14 months of the experiment.

Organic mulch favored sage plant height for 8 to 11 months after establishment, but had no apparent effect on plant survival. Improved plant development of sage treated with organic mulch may be attributed to the contribution of approximately 200 kg of nitrogen ha$^{-1}$ year$^{-1}$ from the mulch, lower soil temperatures, and reduced *M. incognita* J2 population.

Although synthetic mulch had no effect on plant development or survival, it significantly reduced *F. oxysporum* population density, especially with lime.

Galls from *M. incognita* were found on the roots of field grown sage. These findings agree with results obtained from glasshouse pathogenicity studies and previous findings by Moreno et al. (1992). None of the treatments over time provided adequate protection, with 90% plant mortality by the end of the test. Any attempt to produce sage would require the use of disease control practices based on further study of the dieback disease.

Methyl bromide enhanced plant survival during the 2-year study with lavender, with only 6% plant mortality during the first year and 11% total mortality by the end of the second year. No soil amendment effect was detected on lavender plant height. A constant height increase over the study period was consistent between treatments. After one and one-half years, methyl bromide in combination with synthetic mulch showed only 9% root infectation by *F. oxysporum*, compared to 82% root infection for plants in the untreated control.

Treatment of lavender with organic mulch resulted in increased plant height, as compared to height in other treatments for 16 months after establishment, but had no apparent effect on plant survival. As with sage, improved plant development of lavender treated with organic mulch could have resulted from the contribution of approximately 200 kg of nitrogen $ha^{-1}$ $year^{-1}$ from the mulch and generally lower soil temperatures. Spike weight was affected by mulch treatments during the first year. Organic mulch in combination with lime appeared to give the highest yield with 816.5 g $m^{-2}$.

No root-knot galls were observed in lavender. Lack of galls on field-grown lavender corresponds to the results obtained in the combined pathogenicity glasshouse study, in which lavender roots showed little gall formation.

Lavender is not a good host of *M. incognita*. The chemotype used in this research contains 7% of linalool. Linalool is a terpenoid which had been considered by Hasseb et al. (1987) and Chatterjee et al. (1982) to have nematicidal properties.

This study provided evidence that the die-back disease of lavender can be reduced with the use of an integrated control system. Study findings point to *F. oxysporum* as the principle pathogen affecting lavender. Although, not consistently demonstrated by the pathogenicity studies, it appeared that sage is affected by both pathogens, *F. oxysporum* and *M. incognita* race 1.

# APPENDIX A

## GERMPLASMS USED IN THE RESEARCH

Table A-1. Oil composition of sage (*Salvia officinalis* L.) chemotype.[†]

| Constituent | % |
|---|---|
| myrcene | 0.64 |
| limonene | 0.78 |
| 1,8-cineole | 3.81 |
| γ-terpinene | 0.69 |
| ρ-cymene | 1.17 |
| terpinolene | 0.44 |
| α-thujone | 27.94 |
| β-thujone | 6.25 |
| camphor | 29.42 |
| bornyl acetate | 2.82 |
| β-caryophyllene | 5.21 |
| α-humulene | 9.85 |
| borneol | 4.69 |
| viridiflorol | 5.22 |

[†] This germplasm falls into chemotype group # 2 of the classification done by Tucker and Maciarello (1990). This group is characterized by the dominance of the following compounds: camphor>α-thujone>β-thujone> 1,8-cineole. There are two commercial and very important cultivars grown in California which belong to this group. The cultivars 'Tricolor' and 'Woodcote Farm' are produced for fresh and essential oil markets. Although, they do not compete well with the imported sage from Yugoslavia, the political situation in that country has limited production with the result that California sage essential oil production has increased.

Information taken from:
Tucker, A. and M. Maciarello. 1990. The essential oils of of dalmation sage (*Salvia officinalis* L.). Journal of Essential Oil Research 5:139-144.

Table A-2. Oil composition of lavender (*Lavandula* x *hybrida* Balb.ex Ging) chemotype.

| Constituent | % ± S.D.[†] |
|---|---|
| α-pinene | 5.97±0.53 |
| camphene | 1.12±0.12 |
| β-pinene | 7.68±0.48 |
| sabinene | 3.10±0.24 |
| myrcene | 1.38±0.13 |
| α-terpinene | 0.08±0.05 |
| limonene | 2.41±0.45 |
| 1,8-cineole | 33.53±1.69 |
| (E)-ocimene | 0.54±0.14 |
| γ-terpinene | 0.26±0.04 |
| ρ-cymene | 0.16±0.02 |
| terpinolene | 0.21±0.03 |
| n-hexyl butyrate | 0.20±0.03 |
| 1-octen-3-ol | 0.40±0.04 |
| (E)-sabinene hydrate | 0.96±0.11 |
| linalool | 7.04±1.23 |
| camphor | 12.36±0.35 |
| linalyl acetate | 0.03±0.02 |
| (E)-α-bergamotene | 0.51±0.11 |
| pinocarvone | 1.00±0.05 |
| terpinen-4-ol | 0.89±0.26 |
| β-caryophyllene | 0.57±0.18 |
| myrtenal | 1.38±0.34 |
| (E)-pinocarveol | 1.14±0.13 |
| α-terpineol | 3.98±0.32 |
| borneol | 1.10±0.06 |
| germacrene D | 0.50±0.15 |
| β-bisabolene | 0.49±0.11 |
| cuminaldehyde | 0.10±0.01 |
| caryohyllene oxide | 0.30±0.05 |

[†] Percentage of oil and standard deviation.

Information taken from:
Tucker, A., M. Maciarello, S. Angell, J. Espaillat and E. French. 1993. The essential oil of *Lavandula* x *hybrida* Balb. ex Ging., a distinct hybrid from *L.* x *heterophylla* Poir (Labiatae). Journal of Essential Oil Research 5:443-445.

## APPENDIX B

## PROCEDURES USED IN THE LABORATORY

Table B-1. Soil microwaving procedure.

| Step | Procedure |
|---|---|
| 1 | Determine the moisture content in the soil. Take four or five sub-samples of approximately 5 g and weigh them to the third decimal. Dry the sub-samples in a oven at 204ºC for 15 min. Weight the dried soil again and calculate the percent soil moisture. |
| 2 | Bring soil to ± 5 % moisture content. The soil should stay together and not drip water when squeezed between the fingers. |
| 3 | Weigh 1 kg of soil and place it in a 0.04-mm thick polyethylene bag. |
| 4 | With the bag mouth open, place the soil in the Microwave oven for 4 min at high power (625 w heating power; 2,450 Mhz). |
| 5 | After the soil has been microwaved, remove the bag carefully to avoid breaking it. |
| 6 | Put the bags in a clean place with the mouth open to cool to room temperature. After 3 or 4 hours the soil should be cool enough to close the bag and store for future use. |

Procedure adapted from:
Ferriss, R.S. 1984. Effects of microwave oven treatment on microorganisms in soil. Phytopathology 74:121-126.

Table B-2. Procedure for the use of Komada's medium for the selective isolation of *Fusarium oxysporum* from soil.

| Step | Procedure |
|---|---|
| 1 | Add these components for 500 ml of basal medium:<br>1) 10.0 g bacto agar<br>2) 0.25 g $MgSO_47\ H_2O$<br>3) 0.5 g $K_2HPO_4$<br>4) 0.25 g KCL<br>5) 0.005 g Fe (I ml) from Fe-EDTA stock solution.<br>6) 1.0 g L-asparagine<br>7) 10.0 g D-galactose |
| 2 | Bring to 500 ml with deionized water in a 1.0-L flask. |
| 3 | Autoclave for 15 min at 103 megapascal. After removal from the autoclave, let the flask cool to ± 45ºC and add the following:<br>8) 0.5 g PCNB (0.67 gr of 75% a.i. Terrachlor).<br>9) 0.25 g bacto-oxgall.<br>10) 0.5 g $Na_2B_4O_7$-10 $H_2O$.<br>11) 0.15 g (300 ppm) streptomycin sulfate.<br>12) Antifoam (2 or 3 drops). |
| 4 | Adjust pH to 3.8 ± 0.2 with 10% phosphoric acid (1.6 ml of acid routinely used to adjust the medium to about pH 4.2). |
| 5 | Pour ± 15 ml into each petri plate. The prepared amount of medium should be enough for 32 petri plates. |

Procedure taken from:
Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Review of Plant Protection Research 8:114-125.

Table B-3. Procedure for the mass production of conidia of *Fusarium oxysporum* in wheat berries.

| Step | Procedure |
|---|---|
| 1 | Mix the following components in a 1-liter wide mouth flask:<br>1) 200 gr wheat berries<br>2) 200 ml deionized $H_2O$.<br>Allow the mixture to sit for 24 hrs. |
| 2 | Autoclave for 30 mins at 103 megapascal. After removal from the autoclave let the flask cool and sit for 24 hrs. |
| 3 | Autoclave for a second time for 30 mins at 103 megapascal. |
| 4 | After removal from the autoclave let the flask cool. Inoculate the autoclaved flasks with conidia of *F. oxysporum* under the hood. From petri plates containing the isolates to be mass produced, take five to six plugs using a small cork borer. The cork borer and other tools must be alcohol-flamed for disinfestation. Place the plugs with mycelium in the middle of the flask. Before closing the flask, flame disinfect the mouth. |
| 5 | When inoculation has been completed, put the flasks in an incubator at 12 hours light and 12 hours dark. |
| 6 | Promote the rapid colonization of the wheat berries by shaking the flasks every 3 days, making sure the chunks of berries are separated. Repeat this step three or four times. |
| 7 | When the berries are completely colonized, they can be used to inoculate autoclaved soil at a rate of 10 g of wheat berries $kg^{-1}$ of soil. |

Procedure taken from:

Nelson, P.E., T.A. Toussoun, L.W. Burgess, W.F.O. Marasas, and C.M. Liddell. 1986. Isolating, identifying, and producing inoculum of pathogenic species of *Fusarium*. p.54-59 *In* K.D. Hickey (ed.) Methods of evaluating pesticides for control of plant pathogens. American Phytopathological Society Press, St. Paul.

Table B-4. Extraction of juveniles of *Meloidogyne incognita* using the Baermann Funnel method.

| Step | Procedure |
|---|---|
| 1 | Extract roots from soil carefully to avoid damaging roots. |
| 2 | Wash roots with tap water and observe galls and egg mass formation. |
| 3 | Cut out root pieces which contain galls. |
| 4 | Surface disinfest by submerging the roots in 0.5 % NaCl solution for 30 seconds. |
| 5 | Put the roots in ± 20 ml deionized water and massage gently for 1 min to liberate the eggs. |
| 6 | Sieve the root suspension in 200-and 500-mesh sieves to create an egg suspension. |
| 7 | Repeat steps 5 and 6 one more time. |
| 8 | Let the suspension to sit still for 30 min to allow the eggs to go to the bottom. |
| 9 | The egg suspension is then washed onto a scotties® facial tissue on a screen support in a Baermann pan. The suspension is poured onto the paper tissue an spread out to form a thin layer. |
| 10 | Enough water is added to fill the pan to the level of the tissue. |
| 11 | The edges of the tissue are folded in to prevent loss of water by capillarity. |
| 12 | After 5-7 days at ± 24ºC, the tissue with the original root material is discarded and the screen is rinsed into the pan. |
| 13 | The water from the pan is poured into a 400-mesh sieve to catch the hatched eggs and determine the number of juveniles. |

Procedure taken from:
Rodríguez-Kábana, R., and M.H. Pope. 1981. A simple incubation method for the extraction of nematodes from soil. Nematrópica 11:175-186.

Table B-5. Procedure for the extraction of ecto-parasitic nematodes using the centrifugal flotation method.

| Step | Procedure |
|---|---|
| **Preparing the soil:** | |
| 1) | Remove all large pieces of plant material, mix throughly, and fill a 100-ml beaker with packed soil. |
| 2) | Empty soil into a kitchen strainer placed on a 2-liter container. |
| 3) | Wash soil through the strainer with forceful stream of deionized water to agitate the water and suspend soil in the water. Then, allow water to stand for 30 seconds for sand to settle. |
| 4) | Pour suspension onto a 400-mesh sieve, holding the sieve at ± 45º angle to a stream of water. |
| 5) | Wash soil and nematodes from the 400-mesh sieve into a 50-cm$^3$ centrifuge tube by holding the sieve vertically over a funnel and washing from the back with a fine stream of water from a wash bottle. |
| 6) | Fill the tube to 6 mm from the top (this includes rinsing anything which may stick to the funnel). |
| **Centrifuging of the soil:** | |
| 7) | Place eight balanced tubes in the centrifuge; use a blank tube filled with water if an odd number of samples are being run in order to balance the centrifuge. |
| 8) | Run centrifuge at 3,200 RPM for 4 minutes. Nematodes will be in the plug at the bottom of the tubes. |
| 9) | Pour off the supernatant with a smooth twisting motion without disturbing the plug at bottom of tube. Some organic matter residue remaining at collar of tube can be removed with a finger. |
| 10) | Fill the tube half full with sugar solution (454 g liter$^{-1}$ of water) plug the end of the tube with a rubber stopper and shake to resuspend the plug. Then fill tubes to within 12 mm of the top with sugar solution. |
| 11) | Centrifuge as before in step 9. After centrifugation the nematodes will remain in the sugar solution. |
| 12) | Pour solution into a 500-mesh sieve to catch nematodes. |
| **Saving the nematodes:** | |
| 13) | Rinse nematodes with water to remove sugar, then wash nematodes through a funnel into a clean 50-cm$^3$ centrifuge tube or other container for examination. |

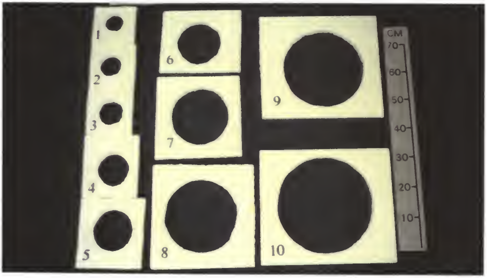
Jenkins, W.R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Disease Reports 48:692.

# APPENDIX C

## RATING SCALES USED TO EVALUATE PATHOGENS EFFECTS

Table C-1. Template used to determine canopy width as an assessment of plant development.†

| Template | Diameter of the Hole |
|---|---|
| | -- cm -- |
| 1 | 6.5 |
| 2 | 7.5 |
| 3 | 8.5 |
| 4 | 11.0 |
| 5 | 13.0 |
| 6 | 15.5 |
| 7 | 19.0 |
| 8 | 23.5 |
| 9 | 27.5 |
| 10 | 31.0 |



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† Individual plants were placed on a stand with a riser which insured that the data was taken at the same height. All branches must pass through the template hole to be considered in that category.

Table C-2. Scale[†] for root rating of *Fusarium oxysporum* effects on roots of sage and lavender.

| Rate | Description | | |
|---|---|---|---|
| | Root color | Adventicious roots | Plant condition |
| 1 | white | no | healthy |
| 2 | white | yes | healthy |
| 3 | white | yes | yellowish (y) |
| 4 | some brown | yes | y + dwarfed |
| 5 | many brown | no | dwarfed + wilted |
| 6 | mostly brown | no | moribund |

[†] This scale was elaborated for the purpose of this research based on the symptoms observed in the preliminary pathogenicity test.

Table C-3. Scale for root-knot gall rating used in the pathogenicity and disease control studies.

| Category | Number of galls per root system |
|---|---|
| 0 | 0 |
| 1 | 1-2 |
| 2 | 3-10 |
| 3 | 11-30 |
| 4 | 31-100 |
| 5 | >100 |

Scale taken from:
Taylor A. and J. Sasser. 1978. Biology, identification and control of root-knot nematodes (*Meloidogyne* spp.). North Carolina State University Graphics, Raleigh, N.C.

Table C-4. Means and ANOVA of the effect of lime treatments on soil pH (April 1992-July 1994).

| Soil Amendment Treatments | 1992 April | 1992 Aug | 1992 Nov | 1993 Dec | 1993 July | 1994 April |
|---|---|---|---|---|---|---|
| Brom-o-gas | 6.0 | - | 6.0 | - | - | 6.2 |
| Lime | 6.0 | 6.2 | 6.1 | 6.4 | 6.6 | 7.2 |
| Unamendmended | 6.0 | - | 5.8 | 6.0 | 6.0 | 6.2 |
| ANOVA:[†] | | | | | | |
| Soil amendment | | | *[‡] | | | * |
| CV (%) | | | 3.12 | | | 2.42 |

[†] ANOVA of a randomized complete block design with four replications.

[‡] * = F value significant at $P \leq 0.05$.

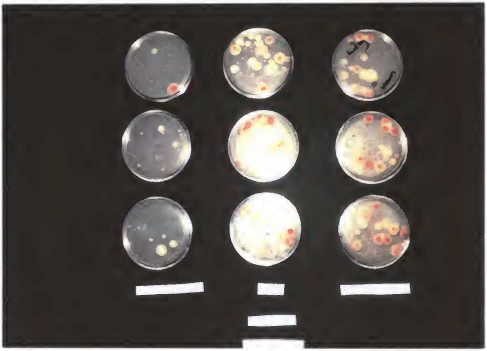

Figure 1. Effect of brom-o-gas soil amendment treatment on the population density of *Fusarium oxysporum* at 2 months after establishment of the disease control studies with sage and lavender.

# APPENDIX D

## DATA COLLECTED IN THE COMBINED PATHOGENICITY TESTS OF SAGE

Table D-1. Dates at which the number of dead plants were recorded.

| Days After Establishment | Year | Test 1[†] | Test 2 |
|---|---|---|---|
| 14 | 1994 | August 12 | August 27 |
| 38 | | September 6 | September 20 |
| 72 | | October 10 | October 24 |
| 99 | | November 7 | November 20 |
| 122(123)[‡] | | December 1 | December 13 |
| 178 | 1995 | January 25 | February 7 |
| 232 | | March 20 | April 2 |
| 291(300) | | May 18 | June 9 |

[†] Test 1 = first pathogenicity test; Test 2 = second pathogenicity test.

[‡] In test 2 the data were collected and plants harvested at a different day after establishment.

Table D-2. Dates at which the plant height data were collected.

| Days After Establishment | Year | Test 1[†] | Test 2 |
|---|---|---|---|
| 2 | 1994 | July 31 | August 15 |
| 44 | | September 11 | September 27 |
| 123 | | November 29 | December 15 |
| 204 | 1995 | February 18 | March 6 |
| 247 | | April 2 | April 18 |
| 287(293)[‡] | | May 12 | June 3 |

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[†] Test 1 = first pathogenicity test; Test 2 = second pathogenicity test.

[‡] In Test 2 the data were collected and plants harvested at a different day after establishment.

Table D-3. Bi-weekly maximum and minimum greenhouse temperatures during the combined pathogenicity tests with sage and lavender.

| Date | | Test 1[†] | | Test 2 | |
|---|---|---|---|---|---|
| Month | bi-week[‡] | Max. | Min. | Max. | Min. |
| | | ºC | | | |
| August, 1994 | 1 | 36.3 | 22.3 | | |
| | 2 | 37.0 | 21.4 | 36.3 | 22.3 |
| September | 1 | 36.3 | 21.0 | 37.9 | 21.9 |
| | 2 | 33.6 | 23.0 | 36.2 | 21.6 |
| October | 1 | 32.5 | 19.5 | 33.3 | 20.5 |
| | 2 | 33.6 | 19.2 | 33.8 | 19.5 |
| November | 1 | 35.5 | 19.1 | 33.9 | 19.8 |
| | 2 | 34.9 | 17.3 | 33.3 | 18.0 |
| December | 1 | 34.1 | 18.6 | 33.3 | 17.7 |
| | 2 | 33.2 | 18.4 | 32.5 | 17.6 |
| January, 1995 | 1 | 33.0 | 19.4 | 32.3 | 17.2 |
| | 2 | 32.7 | 20.2 | 32.1 | 17.1 |
| February | 1 | 35.8 | 16.9 | 32.6 | 17.1 |
| | 2 | 36.9 | 16.8 | 33.8 | 17.9 |
| March | 1 | 37.9 | 17.6 | 34.9 | 17.9 |
| | 2 | 35.9 | 18.1 | 37.2 | 17.8 |
| April | 1 | 38.1 | 17.7 | 38.7 | 18.3 |
| | 2 | Harvest | | 40.0 | 17.8 |
| May | 1 | | | 47.0 | 17.8 |
| | 2 | | | Harvest | |

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[†] Test 1 = first pathogenicity test; Test 2 = second pathogenicity test.

[‡] The daily maximum and minimum temperatures were recorded and the average for 2 weeks was calculated.

Table D-4. Correlation matrix of all variables evaluated at harvest in the first pathogenicity test with sage[†].

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | | | | | | | | | | | | | | |
| 2 | | | | | | | | | | | | | | | |
| 3 | -0.35 | 0.12 | 1.00 | | | | | | | | | | | | |
| 4 | 0.64 | -0.06 | -0.57** | 1.00 | | | | | | | | | | | |
| 5 | -0.28 | 0.16 | 0.20 | 0.19 | 1.00 | | | | | | | | | | |
| 6 | -0.16 | -0.23 | -0.38* | 0.11 | 0.26 | 1.00 | | | | | | | | | |
| 7 | -0.20 | -0.07 | -0.29 | -0.09 | -0.12 | 0.56** | 1.00 | | | | | | | | |
| 8 | 0.63** | 0.07 | -0.07 | 0.05 | -0.30 | -0.14 | -0.11 | 1.00 | | | | | | | |
| 9 | 0.46* | 0.02 | 0.02 | 0.04 | -0.12 | -0.20 | -0.19 | 0.57* | 1.00 | | | | | | |
| 10 | 0.57** | -0.14 | -0.15 | 0.04 | -0.44* | -0.10 | -0.02 | 0.61** | 0.70** | 1.00 | | | | | |
| 11 | 0.79** | -0.09 | 0.07 | 0.17 | -0.05 | -0.37 | -0.40* | 0.45* | 0.66** | 0.61** | 1.00 | | | | |
| 12 | 0.87** | 0.08 | -0.22 | 0.10 | -0.23 | -0.04 | -0.16 | 0.73** | 0.55** | 0.71** | 0.69** | 1.00 | | | |
| 13 | 0.11 | 0.88** | 0.12 | -0.11 | 0.04 | -0.31 | -0.17 | 0.24 | 0.13 | 0.18 | 0.04 | 0.22 | 1.00 | | |
| 14 | -0.29 | 0.08 | 0.42* | -0.17 | 0.04 | -0.42* | -0.40* | -0.12 | 0.23 | -0.07 | 0.05 | -0.25 | 0.14 | 1.00 | |
| 15 | 0.38* | -0.02 | 0.11 | 0.88** | 0.05 | -0.11 | 0.01 | -0.31 | -0.17 | -0.08 | -0.04 | -0.36 | 0.07 | 0.76** | 1.00 |

(n s,* *,*) = non significant, significant at $P \leq 0.01$ and $P \leq 0.05$.

Variables Description:

1- *Fusarium oxysporum* inoculum level
2- *Meloidogyne incognita* inoculum level
3- Dead plants
4- Plant height
5- Stem diameter
6- Shoot dry weight
7- Root dry weight
8- *F. oxysporum* root rating
9- *F. oxysporum* ppg of soil
10- Log (x+1) of ppg of soil
11- Infection-main root
12- Infection-fibrous roots
13- *M. incognita* gall rating
14- Number of J2 100 $ml^{-1}$ of soil
15- Log (x+1) J2 100 $ml^{-1}$ of soil

Table D-5. Correlation matrix of all variables evaluated at harvest in the second pathogenic test with sage†.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | | | | | | | | | | | | | | |
| 2 | | | | | | | | | | | | | | | |
| 3 | 0.00 | 0.00 | 0.00 | | | | | | | | | | | | |
| 4 | 0.01 | -0.09 | 0.00 | 1.00 | | | | | | | | | | | |
| 5 | -0.74** | 0.19 | 0.00 | -0.20 | 1.00 | | | | | | | | | | |
| 6 | -0.06 | -0.35 | 0.00 | 0.59** | 0.02 | 1.00 | | | | | | | | | |
| 7 | 0.17 | 0.16 | 0.00 | 0.10 | 0.02 | 0.37 | 1.00 | | | | | | | | |
| 8 | 0.45* | 0.36 | 0.00 | -0.20 | -0.34 | -0.72** | -0.12 | 1.00 | | | | | | | |
| 9 | 0.32 | 0.38 | 0.00 | -0.01 | -0.09 | 0.27 | 0.54** | 0.54** | 1.00 | | | | | | |
| 10 | 0.48* | -0.14 | 0.00 | -0.02 | -0.30 | 0.01 | 0.51** | 0.51** | 0.59** | 1.00 | | | | | |
| 11 | 0.28 | 0.26 | 0.00 | -0.44* | -0.14 | -0.18 | 0.59** | 0.59** | 0.55** | 0.46* | 1.00 | | | | |
| 12 | 0.85** | 0.07 | 0.00 | -0.11 | -0.56** | 0.10 | 0.60** | 0.61** | 0.45* | 0.62** | 0.38* | 1.00 | | | |
| 13 | 0.24 | 0.86** | 0.00 | -0.02 | 0.01 | 0.15 | 0.56** | 0.56** | 0.41** | 0.14 | 0.29 | 0.35 | 1.00 | | |
| 14 | -0.22 | 0.14 | 0.00 | -0.10 | 0.25 | -0.59** | -0.28 | 0.47* | 0.32 | 0.13 | 0.37 | -0.05 | 0.24 | 1.00 | |
| 15 | -0.16 | 0.32 | 0.00 | -0.17 | 0.21 | -0.63** | -0.22 | 0.48* | 0.25 | -0.04 | 0.27 | 0.07 | 0.39* | 0.77** | 1.00 |

(n s,**,*) = non significant, significant at $P \leq 0.01$ and $P \leq 0.05$.

Variables Description:

1- *Fusaroum oxysporum* inoculum level
2- *Meloidogyne incognita* inoculum level
3- Dead plants
4- Plant height
5- Stem diameter
6- Shoot dry weight
7- Root dry weight
8- *F. oxysporum* root rating
9- *F. oxysporum* ppg of soil
10- Log (x+1) of ppg of soil
11- Infection-main root
12- Infection-fibrous roots
13- *M. incognita* gall rating
14- Number of J2 100 $ml^{-1}$ of soil
15- Log (x+1) J2 100 $ml^{-1}$ of soil

# APPENDIX E

## DATA COLLECTED IN THE COMBINED PATHOGENICITY TESTS WITH LAVENDER

Table E-1. Dates at which the number of dead plants were recorded.

| Days After Establishment | Year | Test 1[†] | Test 2 |
|---|---|---|---|
| 48 | 1994 | September 15 | September 30 |
| 72 | | October 9 | October 24 |
| 101 | | November 7 | November 22 |
| 177(170)[‡] | 1995 | January 19 | January 28 |
| 254(238) | | April 6 | April 1 |
| 291(304) | | May 18 | June 2 |

[†] Test 1 = first pathogenicity test; Test 2 = second pathogenicity test.

[‡] In test 2, the data were collected and plants harvested at a different day after establishment.

Table E-2. Dates at which the plant height data were collected.

| Days After Establishment | Year | Test 1[†] | Test 2 |
|---|---|---|---|
| 2 | 1994 | July 31 | August 15 |
| 45 | | September 12 | September 27 |
| 123 | | November 29 | December 14 |
| 204 | 1995 | February 18 | March 6 |
| 247 | | April 3 | April 18 |
| 294(304)[‡] | | May 18 | June 2 |

[†] Test 1 = first pathogenicity test; Test 2 = second pathogenicity test.

[‡] In test 2, the data were collected and plants harvested at a different day after establishment.

Table E-3. Correlation matrix of all variables evaluated at harvest in the first pathogenicity test with lavender[†].

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | | | | | | | | | | | | | | |
| 2 | | | | | | | | | | | | | | | |
| 3 | 0.00 | -0.11 | 1.00 | | | | | | | | | | | | |
| 4 | -0.01 | -0.30 | 0.25 | 1.00 | | | | | | | | | | | |
| 5 | 0.05 | -0.24 | 0.40** | 0.57** | 1.00 | | | | | | | | | | |
| 6 | 0.15 | -0.33 | 0.19 | 0.19 | 0.25 | 1.00 | | | | | | | | | |
| 7 | -0.15 | -0.35 | 0.49** | 0.62** | 0.80** | 0.33 | 1.00 | | | | | | | | |
| 8 | -0.40* | -0.27 | 0.35 | 0.35 | 0.50** | 0.16 | 0.73** | 1.00 | | | | | | | |
| 9 | 0.31 | 0.19 | -0.22 | -0.46* | -0.55** | -0.06 | -0.63** | -0.57** | 1.00 | | | | | | |
| 10 | 0.61** | -0.08 | 0.03 | 0.15 | 0.07 | 0.06 | 0.15 | -0.04 | 0.15 | 1.00 | | | | | |
| 11 | 0.61** | 0.18 | 0.20 | 0.15 | 0.12 | 0.08 | 0.03 | -0.23 | 0.28 | 0.64** | 1.00 | | | | |
| 12 | 0.73** | 0.02 | 0.11 | 0.06 | 0.09 | 0.37 | -0.08 | -0.25 | 0.06 | 0.43* | 0.53** | 1.00 | | | |
| 13 | 0.81** | -0.01 | 0.01 | 0.08 | 0.08 | 0.40* | -0.07 | -0.34 | 0.02 | 0.56** | 0.65** | 0.86** | 1.00 | | |
| 14 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | |
| 15 | 0.35 | 0.34 | -0.16 | -0.18 | -0.24 | 0.01 | -0.34 | -0.37 | 0.23 | 0.19 | 0.25 | 0.20 | 0.31 | 0.73** | 1.00 |

n s = Correlation value non significant; ** = significant at $P \leq 0.01$; and * = significant at $P \leq 0.05$.

Variables Description:

1- *Fusarium oxysporum* inoculum level
2- *Meloidogyne incognita* inoculum level
3- Dead plants
4- Plant height
5- Canopy width
6- Stem diameter
7- Shoot dry weight
8- Root dry weight
9- *F. oxysporum* root rating
10- *F. oxysporum* ppg of soil
11- Logarithm (x+1) of pp in soil
12- Infection-main root
13- Infection-fibrous roots
14- *M. incognita* gall rating
15- Number J2 100 $ml^{-1}$ of soil

Table E-4. Correlation matrix of all variables evaluated at harvest in the second pathogenicity test with lavender†.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | | | | | | | | | | | | | | |
| 2 | | | | | | | | | | | | | | | |
| 3 | -0.35 | 0.00 | 1.00 | | | | | | | | | | | | |
| 4 | 0.32 | 0.07 | -0.24 | 1.00 | | | | | | | | | | | |
| 5 | 0.11 | 0.14 | -0.18 | 0.87** | 1.00 | | | | | | | | | | |
| 6 | -0.25 | 0.29 | 0.30 | 0.10 | 0.17 | 1.00 | | | | | | | | | |
| 7 | -0.22 | 0.16 | 0.40* | 0.27 | 0.37 | 0.60** | 1.00 | | | | | | | | |
| 8 | -0.16 | 0.18 | 0.49* | -0.03 | 0.09 | 0.66** | 0.44* | 1.00 | | | | | | | |
| 9 | 0.20 | 0.30 | -0.41* | 0.04 | 0.04 | -0.31 | -0.31 | -0.41 | 1.00 | | | | | | |
| 10 | 0.08 | 0.43* | -0.22 | 0.03 | -0.01 | 0.00 | -0.10 | -0.29 | 0.69** | 1.00 | | | | | |
| 11 | 0.24 | 0.23 | -0.35 | 0.05 | -0.03 | -0.22 | -0.16 | -0.66** | 0.60** | 0.74** | 1.00 | | | | |
| 12 | 0.67** | 0.07 | -0.46** | 0.26 | 0.03 | -0.38* | -0.52** | -0.48* | 0.38 | 0.32 | 0.44* | 1.00 | | | |
| 13 | 0.62** | -0.07 | -0.59** | 0.36 | 0.26 | -0.38 | -0.34 | -0.46* | 0.39* | 0.16 | 0.22 | 0.63** | 1.00 | | |
| 14 | 0.24 | 0.24 | -0.09 | -0.23 | -0.23 | -0.06 | -0.39* | -0.03 | 0.07 | 0.07 | 0.13 | 0.18 | 0.13 | 1.00 | |
| 15 | -0.24 | -0.05 | -0.14 | -0.15 | -0.14 | -0.16 | -0.38* | -0.29 | 0.39* | 0.20 | 0.26 | 0.13 | -0.13 | -0.07 | 1.00 |

n s = Correlation value non significant; ** = significant at $P \leq 0.01$; and * = significant at $P \leq 0.05$.

Variables Description:

1- *Fusarium oxysporum* inoculum level
2- *Meloidogyne incognita* inoculum level
3- Dead plants
4- Plant height
5- Canopy width
6- Stem diameter
7- Shoot dry weight
8- Root dry weight
9- *F. oxysporum* root rating
10- *F. oxysporum* ppg of soil
11- Log (x+1) ppg of soil
12- Infection-main root
13- Infection-fibrous roots
14- *M. incognita* gall rating
15- Number J2 100 $ml^{-1}$ of soil

# APPENDIX F
# DATA COLLECTED IN THE DISEASE CONTROL STUDY WITH SAGE

Table F-1. Plant height means in response to soil amendment and mulch treatments.

| Treatments | | Measuring Date | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1992 | | | 1993 | | |
| Soil Amendment | Mulch | 8/20 | 9/17 | 12/8 | 3/19 | 6/9 | 9/15 |
| | | cm[†] | | | | | |
| Methyl bromide | Organic | 5.8 | 18.0 | 33.5 | 35.8 | 38.9 | 22.8 |
| | Synthetic | 5.5 | 14.6 | 26.5 | 28.9 | 32.0 | 18.8 |
| | Nonmulch | 5.3 | 14.6 | 25.4 | 27.9 | 31.7 | 26.1 |
| | $\bar{x}$[‡] | 5.5 | 15.7 | 28.5 | 30.9 | 34.2 | 22.6 |
| Lime | Organic | 5.5 | 12.7 | 21.5 | 27.1 | 34.9 | 29.7 |
| | Synthetic | 5.4 | 11.9 | 20.2 | 23.3 | 26.0 | 17.5 |
| | Nonmulch | 4.9 | 13.1 | 21.1 | 25.7 | 32.1 | 14.7 |
| | $\bar{x}$ | 5.3 | 12.6 | 20.9 | 25.4 | 31.0 | 20.7 |
| Nonamended | Organic | 5.3 | 14.4 | 22.9 | 25.0 | 25.6 | 20.6 |
| | Synthetic | 5.0 | 13.9 | 24.4 | 22.4 | 20.8 | 24.8 |
| | Nonmulch | 5.3 | 14.1 | 24.8 | 25.1 | 19.0 | 19.0 |
| | $\bar{x}$ | 5.2 | 14.1 | 24.0 | 24.2 | 21.8 | 21.5 |

[†] The plant height data are the average of four replications.

[‡] Average of soil amendment treatments across mulch treatments.

Table F-2. Means of sage plant height in response to mulch treatments averaged across soil amendment treatments.

| Mulch Treatment | Measuring Date | | | | | |
|---|---|---|---|---|---|---|
| | 1992 | | | 1993 | | |
| | 8/20 | 9/17 | 12/8 | 3/19 | 6/9 | 9/15 |
| | cm[†] | | | | | |
| Organic | 5.5 | 15.1 | 26.0 | 29.3 | 33.1 | 24.4 |
| Synthetic | 5.3 | 13.5 | 23.7 | 24.9 | 26.3 | 20.4 |
| Nonmulch | 5.2 | 13.9 | 23.8 | 26.2 | 27.6 | 19.9 |

† The plant height data are the average of four replications.

Table F-3. Means of percent dead plants in response to soil amendment and mulch treatments.

| Treatments | | Date of dead plant counts | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1992 | | | | | | | 1993 | | | | |
| Soil Amendment | Mulch | 7/20 | 8/25 | 9/17 | 9/29 | 10/27 | 11/19 | 12/8 | 2/5 | 4/28 | 7/10 | 8/29 | 9/30 |
| | | percent dead plants[†] | | | | | | | | | | | |
| Methyl bromide | Organic | 4.0 | 5.0 | 5.0 | 5.0 | 6.5 | 6.5 | 6.5 | 7.5 | 10.0 | 31.5 | 90.0 | 96.5 |
| | Synthetic | 0.0 | 0.0 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 4.0 | 25.0 | 75.0 | 100.0 |
| | Nonmulch | 5.0 | 5.0 | 5.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 27.5 | 60.0 | 74.0 |
| | $\bar{x}$[‡] | 3.0 | 3.5 | 4.0 | 5.5 | 6.0 | 6.0 | 6.0 | 6.5 | 8.0 | 28.0 | 75.0 | 90.0 |
| Lime | Organic | 4.0 | 11.5 | 20.0 | 31.5 | 37.5 | 42.5 | 44.0 | 56.5 | 52.5 | 65.0 | 90.0 | 99.0 |
| | Synthetic | 4.0 | 5.0 | 6.5 | 15.0 | 22.5 | 30.0 | 35.0 | 37.5 | 50.0 | 54.0 | 85.0 | 94.0 |
| | Nonmulch | 5.0 | 6.5 | 12.5 | 22.5 | 32.5 | 35.0 | 37.5 | 46.5 | 61.5 | 72.5 | 92.5 | 92.5 |
| | $\bar{x}$ | 4.3 | 7.5 | 13.0 | 23.0 | 31.0 | 36.0 | 39.0 | 43.5 | 54.5 | 64.0 | 89.0 | 95.0 |
| Nonamended | Organic | 2.5 | 4.0 | 9.0 | 19.0 | 32.5 | 40.0 | 45.0 | 45.0 | 51.5 | 61.5 | 90.0 | 94.0 |
| | Synthetic | 7.5 | 9.0 | 11.5 | 24.0 | 25.0 | 30.0 | 39.0 | 42.5 | 47.5 | 60.0 | 81.5 | 86.5 |
| | Nonmulch | 7.5 | 10.0 | 14.0 | 26.5 | 36.5 | 50.0 | 51.5 | 54.0 | 59.0 | 75.0 | 94.0 | 97.5 |
| | $\bar{x}$ | 6.0 | 7.5 | 11.5 | 23.0 | 31.5 | 40.0 | 45.0 | 47.0 | 52.5 | 65.5 | 88.5 | 92.5 |

[†] The percent dead plants data are the average of four replications.

[‡] Means response to soil amendment treatment averaged across mulch treatments.

Table F-4. Percent dead plant means inresponse to mulch treatments averaged across soil amendment treatments.

| Mulch Treatment | Date of dead plant count | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1992 | | | | | | | 1993 | | | | |
| | 7/20 | 8/25 | 9/17 | 9/29 | 10/27 | 11/19 | 12/8 | 2/5 | 4/28 | 7/10 | 8/29 | 9/30 |
| | percent dead plants† | | | | | | | | | | | |
| Organic | 4.0 | 5.0 | 5.0 | 5.0 | 6.5 | 6.5 | 6.5 | 7.5 | 10.0 | 31.5 | 90.0 | 96.5 |
| Synthetic | 0.0 | 0.0 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 4.0 | 20.0 | 75.0 | 100.0 |
| Nonmulch | 5.0 | 5.0 | 5.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 27.5 | 60.0 | 74.0 |

† The percent dead plants data is an average of four replications.

Table F-5. Effect of Mulch treatments on soil temperature at the 5-cm depth.

| Mulch Treatment | Rep | Date of soil temperature reading[†] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 1992 | | | | 1993 | | | | |
| | | 8/20 | 9/15 | 10/1 | 12/17 | 2/5 | 4/7 | 6/10 | 8/10 | 9/18[‡] |
| | | ºC | | | | | | | | |
| Organic | 1 | 34.1[§] | 28.3 | 22.8 | 17.9 | 16.8 | 23.3 | 33.8 | 31.2 | 27.9 |
| Synthetic | | 39.0 | 29.1 | 22.1 | 18.7 | 17.8 | 23.7 | 38.1 | 34.7 | 31.6 |
| Nonmulch | | 37.5 | 28.1 | 21.6 | 18.9 | 18.0 | 23.9 | 35.4 | 32.0 | 32.0 |
| Organic | 2 | 36.5 | 28.5 | 22.7 | - | 16.2 | - | 32.5 | 31.0 | 28.6 |
| Synthetic | | 39.8 | 30.0 | 22.7 | - | 18.0 | - | 38.6 | 33.0 | 31.7 |
| Nonmulch | | 38.5 | 29.1 | 22.2 | - | 18.9 | - | 35.1 | 33.6 | 31.1 |
| Organic | 3 | 35.0 | 28.5 | 22.6 | - | 16.5 | - | 34.7 | 32.1 | 28.0 |
| Synthetic | | 40.5 | 29.9 | 23.0 | - | 17.2 | - | 37.3 | 35.5 | 31.5 |
| Nonmulch | | 38.5 | 29.1 | 22.1 | - | 17.8 | - | 36.8 | 33.4 | 31.0 |
| Organic | 4 | 34.3 | 28.6 | 23.1 | - | - | - | - | - | - |
| Synthetic | | 40.7 | 28.6 | 22.7 | - | - | - | - | - | - |
| Nonmulch | | 39.7 | 29.0 | 22.0 | - | - | - | - | - | - |
| Organic | $\bar{x}$ | 35.0 | 28.5 | 22.8 | 17.9 | 16.5 | 23.3 | 33.6 | 31.4 | 28.2 |
| Synthetic | | 40.0 | 29.4 | 22.6 | 18.7 | 17.7 | 23.7 | 40.0 | 34.4 | 31.6 |
| Nonmulch | | 38.6 | 28.8 | 22.0 | 18.9 | 18.2 | 23.9 | 35.8 | 33.0 | 31.4 |

[†] Temperature recording made from 1300 to 1400 hours.

[‡] Data collection was discontinued on Nov. 18, 1993 due to canopy closure, which reduced the effect of mulch treatments on soil temperature.

[§] Soil temperature averaged across the three soil amendment treatments.

Table F.6 Analysis of variance summary of temperature response to mulch treatments at the 5-cm depth.[†]

| Source[§] | 1992 | | | | 1993 | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 8/20 | 9/15 | 10/1 | 12/17 | 2/5 | 4/7 | 6/10 | 8/10 | 9/18 |
| Replications | n s[‡] | n s | n s | - | n s | - | n s | n s | n s |
| Mulch | * * | * | * * | - | n s | - | * * | * | * * |
| CV (%) | 2.11 | 1.45 | 1.16 | | 0.90 | | 1.94 | 2.67 | 1.39 |

[†] ANOVA for a randomized complete block design with four replications in 1992, and three replications in 1993.

[‡] n s = F value non significant; ** = F value significant at $P \leq 0.01$; * = F value significant at $P \leq 0.05$.

[§] Mulch treatments are organic mulch, synthetic mulch and nonmulch.

Table F-7. Effect of Mulch treatments on soil temperature at the 10-cm depth.

| Mulch Treatment | Rep | Date of soil temperature reading[†] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 1992 | | | | 1993 | | | | |
| | | 8/20 | 9/15 | 10/1 | 12/17 | 2/5 | 4/7 | 6/10 | 8/10 | 9/18[‡] |
| | | ºC | | | | | | | | |
| Organic | 1 | 31.9[§] | 27.5 | 22.6 | 17.4 | 15.8 | 22.2 | 32.6 | 30.4 | 26.3 |
| Synthetic | | 36.3 | 28.1 | 22.1 | 18.1 | 16.8 | 22.1 | 36.7 | 33.0 | 30.0 |
| Nonmulch | | 33.4 | 27.1 | 21.6 | 18.1 | 16.2 | 22.2 | 33.8 | 30.7 | 28.9 |
| Organic | 2 | 32.7 | 27.6 | 22.7 | - | 15.6 | - | 32.2 | 30.1 | 26.3 |
| Synthetic | | 36.7 | 28.9 | 22.7 | - | 16.2 | - | 36.8 | 32.1 | 31.1 |
| Nonmulch | | 34.7 | 27.8 | 21.9 | - | 16.0 | - | 34.2 | 31.9 | 28.2 |
| Organic | 3 | 31.9 | 27.5 | 22.8 | - | 15.6 | - | 33.1 | 31.1 | 26.7 |
| Synthetic | | 38.0 | 32.2 | 22.6 | - | 16.8 | - | 35.5 | 34.4 | 30.0 |
| Nonmulch | | 35.8 | 27.9 | 21.8 | - | 16.3 | - | 34.0 | 32.1 | 28.6 |
| Organic | 4 | 32.1 | 27.7 | 22.8 | - | - | - | - | - | - |
| Synthetic | | 37.8 | 29.5 | 22.7 | - | - | - | - | - | - |
| Nonmulch | | 36.1 | 27.7 | 22.0 | - | - | - | - | - | - |
| Organic | $\bar{x}$ | 32.2 | 27.6 | 22.7 | 17.4 | 15.7 | 22.2 | 32.6 | 30.5 | 26.4 |
| Synthetic | | 37.2 | 29.7 | 22.5 | 18.1 | 16.6 | 22.1 | 36.3 | 33.2 | 31.0 |
| Nonmulch | | 35.0 | 27.6 | 21.8 | 18.1 | 16.2 | 22.2 | 34.0 | 31.6 | 28.6 |

[†] Temperature recording made from 1300 to 1400 hours.

[‡] The data collection was discontinued on Nov. 18, 1993 due to canopy closure, which reduced the effect of mulch treatments on soil temperature.

[§] Soil temperature averaged across the three soil amendment treatments.

Table F-8. Analysis of variance summary for temperature response to mulch treatments at the 10-cm depth.†

| Source§ | 1992 | | | | 1993 | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 8/20 | 9/15 | 10/1 | 12/17 | 2/5 | 4/7 | 6/10 | 8/10 | 9/18 |
| Replications | n s‡ | n s | ** | - | n s | - | n s | n s | n s |
| Mulch | ** | * | ** | - | n s | - | ** | ** | ** |
| CV (%) | 2.01 | 3.46 | 0.52 | | 1.64 | | 1.97 | 2.02 | 1.87 |

† ANOVA for a randomized complete block design with four replications in 1992, and three replications in 1993.

‡ n s = F value non significant; ** = F value significant at *P*<0.01; * = F value significant at *P*<0.05.

§ Mulch treatments are organic mulch, synthetic mulch and nonmulch.

Table F-9. Shoot dry weight at harvest (September, 1993).

| Mulch treatment | Soil amendment | | | Average |
|---|---|---|---|---|
| | Methyl bromide | Lime | Nonamended | |
| | ---------------- g† | ------------------ | | |
| Organic | 71.1‡ | 191.6 | 106.0 | 122.9 |
| Synthetic | 90.2 | 42.9 | 56.9 | 63.3 |
| Nonmulch | 47.0 | 23.8 | - | 35.4 |
| Average | 69.4 | 86.1 | 81.5 | |

† Shoots were dried for 2 days at 70ºC in a forced air oven.

‡ Three replications from the field disease control study were harvested. The combination of non amended plus non mulch was not evaluated because all plants in that treatment combination had died by harvest time. The average for non amended plus non mulch was obtained using two treatments.

Table F-10. Analysis of variance summary for shoot dry weight of sage at harvest.[†]

| Source | Probability | Significance |
|---|---|---|
| Replications | | n s[‡] |
| Disease Control treatments | .352 | n s |
| CV (%) | 130.72 | |

† ANOVA as a completely randomized design with three replications because the split plot design effect was lost due to the death of all plants in the treatment combination unamended + unmulch.

‡ n s = F value non significant.

Table F-11. Root dry weight at harvest (September, 1993).

| Mulch treatment | Soil amendment | | | Average |
|---|---|---|---|---|
| | Methyl bromide | Lime | Nonamended | |
| | ---------------- g† | ------------------- | | |
| Organic | 116.5‡ | 166.0 | 171.0 | 151.2 |
| Synthetic | 163.3 | 43.0 | 33.9 | 80.1 |
| Nonmulch | 46.8 | 28.0 | - | 37.4 |
| Average | 108.9 | 79.0 | 102.5 | |

† Roots were dried for two days at 70ºC in a forced air oven.

‡ Three replications from the field disease control study were harvested. The combination of nonamended plus nonmulch was not evaluated because all plants in that treatment combination had died by harvest time. The averages of non amended and non mulch were obtained using only two treatments.

Table F-12. Analysis of variance summary for root dry weight of sage at harvest.[†]

| Source | Probability | Significance |
|---|---|---|
| Replications | | n s[‡] |
| Disease Control treatments | .138 | n s |
| CV (%) | 84.0 | |

† ANOVA as a completely randomized design with three replications because the split plot design effect was lost due to the death of all plants in the treatment combination nonamended plus nonmulch.

‡ n s = F value non significant.

# APPENDIX G

# DATA COLLECTED
# IN THE DISEASE CONTROL STUDY
# WITH LAVENDER

Table G-1. Plant height means in response to soil amendment and mulch treatments during a 2-year disease control study with lavender.

| Treatments | | Measuring Date | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1992 | | | 1993 | | | | 1994 | | |
| Soil Amendment | Mulch | 8/20 | 9/17 | 12/8 | 3/19 | 6/9 | 9/15 | 12/20 | 2/15 | 5/10 | 7/15 |
| | | plant height[†] | | | | | | | | | |
| Methyl bromide | Organic | 16.3 | 19.5 | 21.5 | 29.5 | 38.9 | 41.9 | 45.3 | 46.5 | 48.6 | 51.0 |
| | Synthetic | 15.4 | 18.5 | 22.4 | 28.8 | 36.9 | 42.9 | 46.5 | 48.5 | 51.6 | 55.6 |
| | Nonmulch | 13.7 | 17.4 | 18.6 | 28.3 | 38.6 | 44.4 | 47.3 | 49.6 | 52.4 | 56.4 |
| | $\bar{x}$[‡] | 15.1 | 18.5 | 20.9 | 28.9 | 38.2 | 43.1 | 46.4 | 48.2 | 50.9 | 54.3 |
| Lime | Organic | 16.0 | 19.4 | 24.2 | 32.5 | 42.9 | 48.0 | 51.6 | 53.5 | 55.5 | 58.4 |
| | Synthetic | 12.5 | 15.5 | 19.5 | 25.1 | 32.8 | 37.7 | 42.9 | 45.7 | 48.8 | 53.2 |
| | Nonmulch | 10.6 | 14.1 | 18.9 | 23.1 | 29.8 | 36.6 | 40.0 | 41.4 | 43.9 | 47.2 |
| | $\bar{x}$ | 13.1 | 16.3 | 20.9 | 26.9 | 35.2 | 40.8 | 44.8 | 46.9 | 49.4 | 52.9 |
| Nonamended | Organic | 14.7 | 18.7 | 22.7 | 29.9 | 38.8 | 45.8 | 48.9 | 50.8 | 52.3 | 54.7 |
| | Synthetic | 12.4 | 16.6 | 18.4 | 25.2 | 34.9 | 38.6 | 42.1 | 44.3 | 46.7 | 51.6 |
| | Nonmulch | 11.6 | 15.0 | 18.1 | 25.4 | 34.7 | 40.9 | 44.3 | 46.3 | 48.9 | 52.8 |
| | $\bar{x}$ | 12.9 | 16.8 | 19.7 | 26.9 | 36.1 | 41.8 | 45.1 | 47.1 | 49.3 | 53.0 |

[†] Plant height data are the average of five replications.
[‡] Average of soil amendment treatments across mulch treatments.

Table G-2. Plant height means in response to mulch treatments averaged across soil amendment treatments during the 2-year disease control study with lavender.

| Mulch Treatment | Measuring Date | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1992 | | | 1993 | | | | | | |
| | 8/20 | 9/17 | 12/8 | 3/19 | 6/9 | 9/15 | 12/20 | 2/15 | 5/10 | 7/15 |
| | plant height[†] | | | | | | | | | |
| Organic | 15.7 | 19.2 | 22.8 | 30.7 | 40.2 | 45.3 | 48.6 | 50.3 | 52.2 | 54.7 |
| Synthetic | 13.4 | 16.9 | 20.1 | 26.4 | 34.9 | 39.8 | 43.9 | 46.2 | 49.0 | 53.5 |
| Nonmulch | 11.9 | 15.5 | 18.5 | 25.6 | 34.3 | 40.6 | 43.8 | 45.8 | 48.4 | 52.1 |

[†] Plant height data are the average of five replications.

Table G-3. Percent dead plant means in response to soil amendment and mulch treatments during the first year of disease control study with lavender.

| Treatments | | Date of dead plant count | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1992 | | | | | | | 1993 | | |
| Soil Amendment | Mulch | 7/20 | 8/25 | 9/17 | 9/29 | 10/27 | 11/19 | 12/8 | 2/5 | 4/28 | 7/10 |
| | | percent dead plants† | | | | | | | | | |
| Methyl bromide | Organic | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 2.0 |
| | Synthetic | 2.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 5.0 | 7.0 |
| | Nonmulch | 2.0 | 3.0 | 3.0 | 4.0 | 5.0 | 5.0 | 5.0 | 5.0 | 7.0 | 9.0 |
| | $\bar{x}$‡ | 1.5 | 2.5 | 2.5 | 3.0 | 3.5 | 3.5 | 3.5 | 3.5 | 4.5 | 6.0 |
| Lime | Organic | 13.0 | 19.0 | 21.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 24.0 |
| | Synthetic | 10.0 | 11.0 | 13.0 | 13.0 | 13.0 | 13.0 | 13.0 | 15.0 | 16.0 | 22.0 |
| | Nonmulch | 9.0 | 16.0 | 19.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 21.0 | 22.0 |
| | $\bar{x}$ | 10.5 | 15.5 | 17.5 | 18.5 | 18.5 | 18.5 | 18.5 | 19.0 | 19.5 | 22.5 |
| Nonamended | Organic | 12.0 | 20.0 | 22.0 | 22.0 | 22.0 | 23.0 | 23.0 | 24.0 | 30.0 | 30.5 |
| | Synthetic | 9.0 | 13.0 | 17.0 | 17.0 | 17.0 | 17.0 | 18.0 | 19.0 | 19.5 | 23.5 |
| | Nonmulch | 14.0 | 24.0 | 27.0 | 27.0 | 29.0 | 29.0 | 29.0 | 29.0 | 30.0 | 31.0 |
| | $\bar{x}$ | 11.5 | 19.0 | 22.0 | 22.0 | 22.5 | 23.0 | 23.5 | 24.0 | 26.5 | 28.5 |

† Numbers of dead plants data are an average of five replications.

‡ Mean response to soil amendment treatments averaged across mulch treatments.

Table G-4. Percent dead plants means in response to mulch treatments averaged across soil amendment treatments during the first year of disease control study with lavender.

| Mulch Treatment | Date of dead plant counts | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1992 | | | | | | | 1993 | | |
| | 7/20 | 8/25 | 9/17 | 9/29 | 10/27 | 11/19 | 12/8 | 2/5 | 4/28 | 7/10 |
| | ------------------- percent dead plants† --------------------- | | | | | | | | | |
| Organic | 8.5 | 13.5 | 14.5 | 15.0 | 15.0 | 15.5 | 15.5 | 15.5 | 17.5 | 19.0 |
| Synthetic | 7.0 | 10.5 | 11.5 | 11.5 | 11.5 | 11.5 | 11.5 | 12.5 | 13.5 | 17.5 |
| Nonmulch | 8.5 | 14.5 | 16.5 | 17.0 | 18.0 | 18.0 | 18.0 | 18.0 | 19.5 | 20.5 |

† The percent dead plants is an average of five replications.

Table G-5. Percent dead plants means in response to soil amendment and mulch treatments during the second year of disease control study with lavender.

| Treatments | | Date of dead plant counts | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 1993 | | | 1994 | | | |
| Soil Amendment | Mulch | 8/29 | 9/30 | 12/20 | 2/15 | 5/10 | 7/15 | 9/10 |
| | | -------- percent dead plants[†] -------- | | | | | | |
| Methyl bromide | Organic | 2.0 | 3.0 | 3.0 | 3.0 | 3.0 | 4.0 | 4.0 |
| | Synthetic | 11.0 | 16.5 | 16.5 | 16.5 | 16.5 | 17.5 | 17.5 |
| | Nonmulch | 11.0 | 12.0 | 12.0 | 12.0 | 12.0 | 12.0 | 12.0 |
| | $\bar{x}$[‡] | 8.0 | 10.5 | 10.5 | 10.5 | 10.5 | 11.0 | 11.0 |
| Lime | Organic | 24.0 | 25.0 | 25.0 | 25.0 | 25.0 | 25.0 | 25.0 |
| | Synthetic | 24.0 | 26.0 | 26.0 | 26.0 | 26.0 | 27.0 | 27.0 |
| | Nonmulch | 25.0 | 27.0 | 27.0 | 27.0 | 27.0 | 27.0 | 28.0 |
| | $\bar{x}$ | 24.5 | 26.0 | 26.0 | 26.0 | 26.0 | 26.5 | 26.5 |
| Nonamended | Organic | 32.5 | 36.0 | 36.0 | 36.0 | 36.0 | 36.0 | 36.0 |
| | Synthetic | 25.5 | 28.0 | 28.0 | 28.0 | 28.0 | 29.0 | 30.0 |
| | Nonmulch | 32.0 | 34.0 | 34.0 | 34.0 | 34.0 | 34.0 | 34.0 |
| | $\bar{x}$ | 30.0 | 32.5 | 32.5 | 32.5 | 32.5 | 33.0 | 33.5 |

[†] Percent of dead plants is an average of five replications.

[‡] Mean response to soil amendment treatments averaged across mulch treatments.

Table G-6. Percent dead plants means in response to mulch treatments averaged across soil amendment treatments during the second year of disease control study with lavender.

| Mulch Treatment | Date of dead plant counts | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1993 | | | 1994 | | | |
| | 8/29 | 9/30 | 12/20 | 2/15 | 5/10 | 7/15 | 9/10 |
| | ------------ percent dead plants[†] ------------ | | | | | | |
| Organic | 19.5 | 21.5 | 21.5 | 21.5 | 21.5 | 21.5 | 21.5 |
| Synthetic | 20.0 | 23.5 | 23.5 | 23.5 | 23.5 | 24.5 | 25.0 |
| Nonmulch | 22.5 | 24.5 | 24.5 | 24.5 | 24.5 | 24.5 | 24.5 |

† Percent dead plants is an average of five replications.

Table G-7. Effect of mulch treatments on soil temperature at depth of 5 cm in the disease control study with lavender.

| Mulch Treatment | Rep | Date of soil temperature reading[†] | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1992 | | | | | 1993 | | | | | |
| | | 8/20 | 9/15 | 10/1 | 11/19 | 12/17 | 2/5 | 4/7 | 6/10 | 8/10 | 9/18 | 10/18[‡] |
| | | ºC | | | | | | | | | | |
| Organic | 1 | 34.5[§] | 21.0 | 21.4 | 23.9 | 18.3 | 16.8 | 21.6 | 31.0 | 28.7 | 29.6 | 24.5 |
| Synthetic | | 37.8 | 21.4 | 22.2 | 24.3 | 18.5 | 16.3 | 22.0 | 33.7 | 29.8 | 32.7 | 25.2 |
| Nonmulch | | 36.4 | 20.5 | 21.9 | 24.2 | 18.6 | 17.1 | 22.2 | 30.1 | 29.0 | 31.5 | 24.5 |
| Organic | 2 | 35.1 | 21.5 | 22.1 | 23.6 | - | 16.5 | - | 30.2 | 29.3 | 29.5 | 24.9 |
| Synthetic | | 36.8 | 21.4 | 22.7 | 25.4 | - | 16.4 | - | 33.9 | 29.8 | 32.0 | 25.7 |
| Nonmulch | | 37.7 | 20.3 | 22.4 | 26.0 | - | 17.3 | - | 31.5 | 29.2 | 32.7 | 25.1 |
| Organic | 3 | 33.4 | 21.9 | 22.3 | 23.4 | - | 16.8 | - | 29.8 | 29.8 | 30.5 | 25.7 |
| Synthetic | | 36.6 | 21.9 | 23.7 | 27.1 | - | 16.1 | - | 34.1 | 30.4 | 32.4 | 26.7 |
| Nonmulch | | 38.2 | 21.1 | 22.7 | 25.6 | - | 17.4 | - | 29.7 | 30.6 | 32.5 | 25.6 |
| Organic | 4 | 34.2 | 22.0 | - | - | - | - | - | - | - | - | - |
| Synthetic | | 39.0 | 21.8 | - | - | - | - | - | - | - | - | - |
| Nonmulch | | 37.3 | 21.2 | - | - | - | - | - | - | - | - | - |

continued...

Table G-7. continued...

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Organic | 5 | 34.3 | 22.4 | - | - | - | - | - | - | - | - | - |
| Synthetic | | 39.0 | 21.8 | - | - | - | - | - | - | - | - | - |
| Nonmulch | | 37.3 | 21.2 | - | - | - | - | - | - | - | - | - |
| Organic | $\bar{x}$ | 34.3 | 21.8 | 21.9 | 23.6 | 18.3 | 16.7 | 21.6 | 30.1 | 29.3 | 29.9 | 25.0 |
| Synthetic | | 37.8 | 21.7 | 22.8 | 25.6 | 18.5 | 16.3 | 22.0 | 33.9 | 30.0 | 32.4 | 25.9 |
| Nonmulch | | 37.4 | 20.9 | 22.3 | 25.3 | 18.6 | 17.3 | 22.2 | 30.8 | 29.6 | 32.2 | 25.1 |

† Temperature recording was made from 1300 to 1400 hours.
‡ Data collection was discontinued on Nov. 18, 1993 due to canopy closure, which reduced the effect of the mulch treatments on soil temperature.
§ Soil temperature averaged across the three soil amendment treatments.

Table G-8. Analysis of variance summary for temperature response at the depth of 5 cm to mulch treatments.†

| Variable | 1992 | | | | | 1993 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 8/20 | 9/15 | 10/1 | 11/19 | 12/17 | 2/5 | 4/7 | 6/10 | 8/10 | 9/18 | 10/18 |
| Replications | n s‡ | * * | * * | n s | - | n s | - | n s | * | n s | * * |
| Mulch | * * | * * | * | n s | - | n s | - | * | ¥ | * * | * * |
| CV (%) | 2.57 | 0.98 | 1.07 | 3.76 | - | 2.65 | - | 1.87 | 1.06 | 1.75 | 0.55 |

† ANOVA for a randomized complete block design with five and three replications.
‡ n s = F value non significant; ** = F value significant at $P \leq 0.01$; * = F value significant at $P \leq 0.05$; ¥ = F value significant at $P \leq 0.10$.

Table G-9. Effect of mulch treatments on soil temperature at a depth of 10 cm.

| Mulch Treatment | Rep | Date of soil temperature reading[†] | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1992 | | | | | 1993 | | | | | |
| | | 8/20 | 9/15 | 10/1 | 11/19 | 12/17 | 2/5 | 4/7 | 6/10 | 8/10 | 9/18 | 10/18[‡] |
| | | ºC | | | | | | | | | | |
| Organic | 1 | 31.2[§] | 21.6 | 20.9 | 22.0 | 17.7 | 15.1 | 20.9 | 29.3 | 28.1 | 28.7 | 23.5 |
| Synthetic | | 34.7 | 21.8 | 21.2 | 22.3 | 17.9 | 15.3 | 21.7 | 32.7 | 28.4 | 31.2 | 24.6 |
| Nonmulch | | 33.4 | 20.8 | 20.9 | 22.4 | 17.9 | 15.8 | 21.1 | 30.2 | 28.0 | 29.9 | 23.6 |
| Organic | 2 | 31.9 | 22.1 | 21.4 | 21.7 | - | 15.5 | - | 28.8 | 28.1 | 28.6 | 24.0 |
| Synthetic | | 34.8 | 21.8 | 21.9 | 23.7 | - | 15.4 | - | 33.0 | 28.9 | 30.9 | 24.8 |
| Nonmulch | | 34.0 | 20.6 | 21.2 | 23.6 | - | 15.2 | - | 30.6 | 28.4 | 31.2 | 24.2 |
| Organic | 3 | 31.0 | 22.5 | 21.6 | 21.5 | - | 15.4 | - | 29.1 | 28.3 | 29.4 | 24.7 |
| Synthetic | | 34.4 | 22.2 | 22.5 | 25.8 | - | 15.1 | - | 32.8 | 29.6 | 31.2 | 26.0 |
| Nonmulch | | 34.5 | 21.2 | 21.6 | 23.8 | - | 15.5 | - | 30.3 | 29.2 | 30.4 | 24.4 |
| Organic | 4 | 31.5 | 22.4 | - | - | - | - | - | - | - | - | - |
| Synthetic | | 36.3 | 22.4 | - | - | - | - | - | - | - | - | - |
| Nonmulch | | 34.2 | 21.1 | - | - | - | - | - | - | - | - | - |

continued...

Table G-9. continued...

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Organic | 5 | 31.6 | 22.4 | - | - | - | - | - | - | - | - | - |
| Synthetic | | 35.5 | 21.8 | - | - | - | - | - | - | - | - | - |
| Nonmulch | | 35.0 | 21.2 | - | - | - | - | - | - | - | - | - |
| Organic | $\bar{x}$ | 31.4 | 22.2 | 21.3 | 21.7 | 17.7 | 15.3 | 20.9 | 29.1 | 28.2 | 28.9 | 24.1 |
| Synthetic | | 35.1 | 22.0 | 21.8 | 23.9 | 17.9 | 15.3 | 21.7 | 32.8 | 29.0 | 31.1 | 25.1 |
| Nonmulch | | 34.2 | 21.0 | 21.2 | 23.2 | 17.9 | 15.5 | 21.1 | 30.4 | 28.5 | 30.5 | 24.1 |

† Temperature recording was made from 1300 to 1400 hours.

‡ Data collection was discontinued on Nov. 18, 1993 due to canopy closure, which reduced the effect of the mulch treatments.

§ Soil temperature averaged across the three soil amendment treatments.

Table G-10. Analysis of variance summary for soil temperature response to mulch treatments at a depth of 10 cm.[†]

| Variable | 1992 | | | | | 1993 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 8/20 | 9/15 | 10/1 | 11/19 | 12/17 | 2/5 | 4/7 | 6/10 | 8/10 | 9/18 | 10/18 |
| Replications | n s[‡] | * | n s | n s | - | n s | - | n s | * | n s | * * |
| Mulch (b) | * * | * * | * | n s | - | n s | - | ¥ | ¥ | * * | * * |
| CV (%) | 1.55 | 0.91 | 0.85 | 4.49 | - | 2.08 | - | 1.91 | 1.02 | 1.69 | 1.04 |

† ANOVA for a randomized complete block design with five and three replications.

‡ n s = F value non significant; ** = F value significant at $P \leq 0.01$; * = F value significant at $P \leq 0.05$; ¥ = F value significant at $P \leq 0.10$.

Table G-11. Weight of lavender spikes in the first harvest of the disease control study with lavender (June 1993).

| Mulch treatment | Soil amendment | | | Average |
|---|---|---|---|---|
| | Methyl bromide | Lime | Nonamended | |
| | ------------ $g\ m^{-2}$† --------------- | | | |
| Organic | 534.1‡ | 816.5 | 619.0 | 656.5 |
| Synthetic | 440.4 | 388.8 | 587.7 | 472.3 |
| Nonmulch | 586.3 | 465.1 | 518.3 | 523.2 |
| Average | 520.3 | 556.8 | 575.0 | |

† Spikes were dried for 2 days at 70ºC in a forced air oven.

‡ Spike weight is an average of four replications.

Table G-12. Length of lavender spikes in the first harvest of the disease control study with lavender (June 1993).

| Mulch treatment | Soil amendment | | | Average |
|---|---|---|---|---|
| | Methyl bromide | Lime | Nonamended | |
| | ------- spike length in cm† -------- | | | |
| Organic | 38.6‡ | 40.3 | 46.0 | 41.6 |
| Synthetic | 37.5 | 40.2 | 45.4 | 41.0 |
| Nonmulch | 43.9 | 42.7 | 42.9 | 43.2 |
| Average | 40.0 | 41.0 | 44.8 | |

† The largest 20 spikes in each replication were measured from the last leaf to the tip of the spike and the average length was calculated.

‡ Spike lenhth is an average of four replications.

Table G-13. Weight of lavender spikes in the second harvest of the disease control study with lavender (June 1994).

| Mulch treatment | Soil amendment | | | Average |
|---|---|---|---|---|
| | Methyl bromide | Lime | Nonamended | |
| | $g\ m^{-2}$† | | | |
| Organic | 450.1‡ | 559.8 | 394.5 | 468.1 |
| Synthetic | 643.9 | 510.7 | 478.9 | 544.5 |
| Nonmulch | 557.9 | 576.6 | 543.3 | 472.3 |
| Average | 550.7 | 549.0 | 472.3 | |

† Spikes were dried for 2 days at 70ºC in a forced air oven.

‡ Spike weight is an average of five replications.

Table G-14. Length of lavender spikes in the second harvest of the disease control study with lavender (June 1994).

| Mulch treatment | Soil amendment | | | Average |
|---|---|---|---|---|
| | Methyl bromide | Lime | Nonamended | |
| | spike length in cm† | | | |
| Organic | 32.8‡ | 33.8 | 31.9 | 32.8 |
| Synthetic | 33.7 | 34.8 | 34.9 | 34.5 |
| Nonmulch | 34.2 | 35.8 | 36.3 | 35.5 |
| Average | 33.6 | 34.8 | 34.4 | |

† The largest 20 spikes in each replication were measured from the last leaf to the tip of the spike and the average length was calculated.

‡ Spike lenght is an average of five replications.

Table G-15. Gall rating of lavender roots infected with *Meloidogyne incognita* in April, 1994.

| Mulch treatment | Soil amendment | | | Average |
|---|---|---|---|---|
| | Methyl bromide | Lime | Nonamended | |
| | ------------ Gall rating† ------------- | | | |
| Organic | 0‡ | 0 | 0 | 0 |
| Synthetic | 0 | 0 | 0 | 0 |
| Nonmulch | 0 | 0 | 0 | 0 |
| Average | 0 | 0 | 0 | |

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† Gall rating: 0 = 0, 1 = 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100, 5 = >100 galls per plant.

‡ One replication from the field disease control study was sampled. No ANOVA was conducted.

Table G-16. Shoots dry weight of lavender in April, 1994.

| Mulch treatment | Soil amendment | | | Average |
|---|---|---|---|---|
| | Methyl bromide | Lime | Nonamended | |
| | -------------- g† plant | --------------- | | |
| Organic | 463‡ | 890 | 870 | 741 |
| Synthetic | 436 | 854 | 138 | 476 |
| Nonmulch | 435 | 654 | 588 | 559 |
| Average | 445 | 799 | 532 | |

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† Shoots were dried for 2 days at 70ºC in a forced air oven.

‡ One replications from the field disease control study was sampled. No ANOVA was conducted.

Table G-17. Root dry weight of lavender in April, 1994.

| Mulch treatment | Soil amendment | | | Average |
|---|---|---|---|---|
| | Methyl bromide | Lime | Nonamended | |
| | -------------- g† plant | --------------- | | |
| Organic | 155‡ | 299 | 203 | 219 |
| Synthetic | 68 | 163 | 41 | 91 |
| Nonmulch | 127 | 163 | 129 | 140 |
| Average | 117 | 208 | 124 | |

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† Roots were dried for 2 days at 70ºC in a forced air oven.

‡ One replication from the field disease control study was sampled. No ANOVA was conducted.

Table G-18. Stem diameter of lavender in April, 1994.

| Mulch treatment | Soil amendment | | | Average |
|---|---|---|---|---|
| | Methyl bromide | Lime | Nonamended | |
| | ---------------- mm[†] ---------------- | | | |
| Organic | 47.9[‡] | 63.3 | 52.4 | 54.5 |
| Synthetic | 33.1 | 64.1 | 26.6 | 41.3 |
| Nonmulch | 31.5 | 41.0 | 46.5 | 39.7 |
| Average | 37.5 | 56.1 | 41.8 | |

[†] Stem diameter was measured at the soil line using a caliper.

[‡] One replication from the field disease control study was sampled. No ANOVA was conducted.

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# BIOGRAPHICAL SKETCH

José Rafael Espaillat was born on 1 April 1955, in Santiago, The Dominican Republic. In December 1977 he received the Agronomist degree from the Panamerican Agricultural School, Honduras.

From 1978 to 1984 he worked in the private and public agricultural sectors of The Dominican Republic. Working experience included assistance in production and management of sugarcane, papaya, passion fruit, pineapple, cereals and winter vegetables. Also, experience in elaboration and evaluation of agricultural projects was acquired while working for the Interamerican Institute of Agricultural Cooperation (IICA).

In 1985, while he was head of the National Corn Program, the Maize and Wheat International Improvement Center (CIMMYT) awarded him a fellowship to complete undergraduate studies at the University of Florida. In April 1987 he finished his Bachelor of Science in Agriculture degree in agronomy at UF with a certificate in integrated pest management and plant protection.

In August 1988 The Organization of American States (OAS) awarded him a scholarship to do study for a Master of Science Degree. His studies were concentrated in multiple cropping and minimum tillage with a minor in soil science. His thesis research dealt with the development of synthetic corn varieties for sustainable agricultural systems.

Throughout his professional career Mr. Espaillat has been concerned by the constraints in food production and the role agronomists will have to play when looking for solutions within the context of sustainable development. He is a member of the Crop Science Society, American Society of Agronomy, and other regional professional organizations. Also, during his studies at the University of Florida he has been on the dean's list at UF, the National Dean's list, initiated into the Gamma Sigma Delta honor society of agriculture and received 12 awards and certificates of recognition for participation in scientific meetings.

In August 1990, José was awarded an assistantship from the Agronomy Department of the University of Florida to study for a Ph.D. His studies were concentrated in ecology and production of herbs and spices with minors in plant pathology and nematology. Research conducted included vegetative propagation, herbicide screening and germplasm evaluations. His dissertation dealt with the assessment and management of a die-back disease affecting sage and lavender.

He is married to Rosemary Vega de Espaillat and has a 12-year-old son named José Arturo and a 6-year-old daughter named Nicole Marie.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Sherlie H West

Sherlie H. West, Chairman
Professor of Agronomy

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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